JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVII WASHINGTON, D. C., MARCH 15, 1924 NO. 11

STUDIES ON THE POTATO TUBER[1]

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INTRODUCTION

A potato tuber is morphologically a modified stem with its axis greatly shortened and its lateral members only weakly developed, the latter forming what are known as the "potato eyes."

The tuber originates as a stolon from an axillary bud of the underground stem. At first clavate, the young organ soon assumes the shape characteristic of the variety. After a brief period of radial expansion, rapid apical growth becomes evident, new leaf scales are progressively differentiated from the vegetative cone, and in the axils of these scales new eyes and buds develop to maturity. Simultaneously with apical growth intercalary stretching occurs, whereby the spaces between the nodes widen and the spiral arrangement of the eyes becomes more distinct.

The close relationship between tuber and stem, already evident from the grosser morphological features, becomes incontestable when the anatomical structure and the ontogeny of the tissues are studied. In their early development both tuber and stem show practically the same organization; both have a bicollateral stele limited externally by a well developed cortex, internally by the pith. In their further development, however, each organ differentiates in accordance with the rôle which it is destined to play in the life of the plant. The stem, primarily an organ for support, develops a large amount of mechanical tissue and cells for water conduction—its vascular cylinder becomes hard and woody. The tuber, on the other hand, developing under the protective covering of the soil and destined to become an organ for storage, gives rise, as will be shown later, to a large amount of unspecialized parenchyma tissue. The different rôle of these two organs as indicated by their structural make-up is destined conversely to affect their outward appearance; that is, the stem will grow to a considerable length and remain slender, while the tuber will expand laterally as the flow of organic food material, constantly moving downward from the leaves, furnishes the Bausteine for new cells and tissues and for reserve starch which fills the cells as soon as they differentiate and mature.

STRUCTURE OF THE STOLON PRIOR TO TUBER FORMATION

A median section through a stolon tip shows close behind the growing region three distinct tissue zones—the dermatogen, the procambium, and the fundamental meristem. These tissues become greatly extended and differentiated as the stolon tip develops into a tuber.

The dermatogen of the growing point becomes a single-layered epidermis. In surface view its young cells are square or hexagonal and more or less isodiametric. In maturing the cells become stretched in the

[1] Accepted for publication Nov. 24, 1923.

axial direction. Near the apex the cells are radially elongated; the tangential walls are short and equal in extent to the vertical walls. More distant from the growing point the tangential dimension of the cells increases, while the depth remains about the same. The tangential walls of the cells are slightly arched; the outer wall is always thicker than the inner, and is covered with a thin cuticle. Some of the epidermal cells are specialized to form guard cells of the stomates; others enlarge and divide to form cover hairs. Both stomates and hairs, however, are only sparingly found on the young stolon tip, and, because of subsequent development of the periderm, they have only a temporary existence. The young epidermal cells are devoid of chlorophyll and crystals, but rich in protein and solanin. The latter, a glucosidal alkaloid, is also found abundantly in the meristematic regions of the eyes at the time of renewal of growth in the spring.

The cells of the fundamental meristem enlarge without specialization, the region external to the procambium becomes the cortex, the zone inclosed by it becomes the pith.

The cortical parenchyma of the young stolon forms a comparatively broad band of tissue, about 9 cells wide and 345 microns in thickness. The typical cortical cell is rounded polygonal, slightly elongated or isodiametric; its two diameters are, on the average, 81 and 62 microns, respectively. Near the outer periphery the cells are sometimes collenchymatous and have a greater vertical extent. The cells at the inner margin are smaller and grade into the endodermis. Farther from the growing region the cells elongate vertically, but often remain isodiametric or become broader than deep. After the differentiation of the cortical cells from the meristem of the growing region, intercellular spaces form between them. These spaces are very narrow and appear in a cross section as small triangles.

Simultaneous with the formation of these intercellular spaces is the appearance of the first visible storage product—starch. The first starch, usually in the nature of fine round grains, is found in the cells near the procambium; later in larger deposits in other cortical cells. In the peripheral cells of the cortex, which are usually devoid of starch, protein crystals in the form of cubes of varying size are occasionally found; in the mature tuber they occur abundantly. Other cells of the cortex are conspicuous by a dark deposit which fills the entire lumen. This deposit is granular and consists of small crystals of calcium oxalate embedded in the nitrogenous remains of the protoplast of the cell.

The pith of the young stolon is small compared to the area of the cortex. The individual cells are vertically elongated and have a slightly smaller diameter than the cells of the cortex. Both pith and cortical cells are profusely pitted; the pits are minute and arranged in characteristic groups. Intercellular spaces of the nature found in the cortex appear close behind the growing region; starch and crystals, however, are deposited only at a later period.

The procambium, the progenitor of the vascular tissue, forms a hollow cylinder with small projections into the pith. It is composed of small, elongated, thin-walled cells with abundant protoplasm. In their further development these cells either merely enlarge or become highly specialized, forming the elements of the vascular system. The first specialization in the procambium becomes evident in the region of the procambium projections, for, if a cross section of the stolon be examined prior to tuber formation, several groups of vascular tissue with gaps separating

them are readily recognized. The groups of vascular tissue form bicollateral bundles. The xylem, occupying the middle of the bundle, stands out by the larger size of its elements and the secondary thickenings of the cell walls. About equidistant from the xylem internally and externally are small groups of thin-walled cells—the phloem. The outer phloem groups form a more or less continuous band, limited externally by the endodermis and separated from one another by parenchymatous cells of irregular size—the pericycle. The inner phloem groups are separated from the xylem by thin-walled cells which in their entirety make up the *Markkrone*. Interjacent to the outer phloem and the xylem there is a single layer of procambium still in meristematic condition. It becomes the cambium of the bundles and later gives rise to secondary tissue.

The entire xylem of the bundles is limited to a few annular and spiral elements surrounded by unspecialized parenchyma. (Fig. 1.) The first formed protoxylem elements occur surprisingly close to the apex (Pl. 3) in a region in which there is much change and enlargement in the radial and tangential direction; these cells must therefore be able to accommodate themselves to increase in size and change in shape. This is made possible by their structure. The secondary wall is not laid down uniformly over the entire surface of the primary wall, but instead the secondary thickenings consist of rings or spirals. Elongation of the surrounding tissue causes the rings to be pulled farther apart, and as the stretching goes on the cells of the protoxylem may become completely flattened until the lumen is almost closed. The later formed protoxylem elements have thickenings in the form of close spirals and scalariform bands. These elements are shorter than those formed earlier and are evidently developed from more mature initials which had completed their longitudinal growth. The end walls of the protoxylem elements are sloping, occasionally strictly transverse. The elements communicate with each other through a single large pore. (Pl. 2, E, D.)

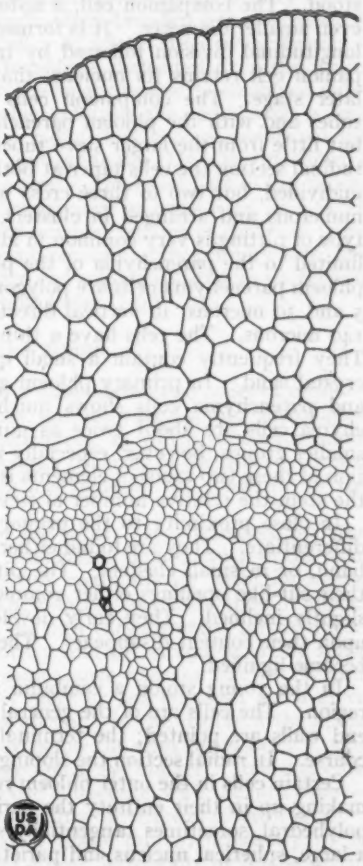

FIG. 1.—Cross section of stolon tip. × 375. (Drawing is exact copy of photomicrograph.)

The phloem groups consist of sieve tubes, their companion cells, and thin-walled parenchyma. The sieve tube is a long cylindrical cell with

end walls strictly transverse. In diameter it varies between 2 and 9 microns, in length between 110 and 250 microns. Wherever branching of the bundles and anastomosis occur, the sieve-tube segments are short and somewhat broader. The sieve tube has a thin protoplasmic lining and albuminous content in which small starch grains are occasionally embedded. After fixation and staining, the content appears as a deep-staining shrunken mass—the slime plug. Its nature is not understood. The companion cell, a sister element of the sieve tube, has an even smaller diameter. It is formed from the sieve-tube mother cell by longitudinal division followed by transverse divisions. Only the companion cell retains its nucleus; that of the sieve tube disappears at a later stage. The companion cells are heavily pitted with the sieve tubes and with the phloem parenchyma. The cells of the latter differ but little from the larger sieve tubes in a cross-sectional cut. In longitudinal section the cells taper at both ends. They do not always remain undivided, but two to three cross walls are formed. The pits are very numerous and arranged in clusters, simulating sieve plates. But this type of pitting is very common in all parenchyma cells and by no means limited to the parenchyma of the phloem groups. In cross section the phloem parenchyma cells are polygonal with a diameter varying between 5 and 30 microns; in vertical direction they may attain a length up to 140 microns. The cells have a thin layer of protoplasm and a nucleus. They frequently contain a small quantity of starch and occasionally crystal sand. In primary phloem groups the proportion of sieve tubes and parenchyma cells shows much variation. Ordinarily the parenchyma cells are about twice as numerous as the sieve tubes. In the smaller groups, however, especially in the outer phloem, the representation of these two kinds of elements is often about equal. Compared with the stem the relative number of sieve tubes in a group is larger.

In close proximity to the primary phloem groups the phloem fibers differentiate. They are found either solitary, in the form of a tangential band, or in small clusters. The pits of these cells are much reduced, their slit-like openings either commonly parallel to the vertical axis or slightly inclined. They early develop a heavy secondary wall, whereupon their content disappears. The secondary wall, however, does not become lignified.

In the young stolon a cambium is noticeable only in the fascicular region. The cells are of the general shape and size of a tracheid. The end walls are pointed; the terminal walls follow an oblique tangential course. In radial section the sloping character of the wall is not evident.

Certain cells in the outer phloem region enlarge without specialization, making up in their entirety the pericycle of the stele. These cells are polyhedral, sometimes tangentially stretched. The young cells contain a large, spherical nucleus and parietal protoplasm. Before long, starch grains are deposited, and in the further growth of the tuber these cells enlarge and multiply rapidly, forcing the phloem groups farther and farther apart. In the mature tuber, as will be seen later, the tissue outside the vascular ring is mostly pericyclic tissue in which numerous phloem groups are scattered. The procambium cells between inner phloem and protoxylem develop in a similar manner. Their rapid multiplication causes a deflection of the inner phloem groups centripetally. As the tuber develops, the differences between cells of the *Markkrone*

and of the pith gradually disappear until it becomes impossible to differentiate between these two tissues.

Between the vascular tissue and the cortex, there is differentiated in close proximity to the apex a special layer of parenchymatous cells—the endodermis. The cells of this layer are smaller than those of the cortex, are more regular, and lack intercellular spaces. On the radial walls there are hyaline swellings which stain red with phloroglucin and hydrochloric acid. These—the *Casparian strips*—though usually found as a narrow band along the radial walls often broaden out and cover a part of the tangential surface of the cell. At a later stage in the development of the endodermis a suberin lamella is deposited over the inner wall, which makes the cells more or less impermeable to dissolved substances and gases. As the young tuber enlarges, the endodermis is less readily detected. The cells with the radial dots become separated by cells resembling those of the cortex, and gradually this layer disappears altogether, and only vestiges of it are seen in the basal part of the tuber. By virtue of its structure the endodermis may be considered as a layer of cells which regulates diffusion between two regions of different pressure, and may be the cause of different pressure. Its primary function is probably to restrict passage of water and soluble substances to certain channels and to aid in the absorption of water (*13*).[2]

THE MATURE TUBER

A median longitudinal cut through a tuber shows two zones of tissue—a central pith with its lateral branches, and the vascular ring surrounded on either side by thick storage parenchyma, in which numerous small islets of phloem are embedded. The outer covering of the tuber is formed by the periderm, a new tissue which has replaced the epidermis of the tuber primordium.

The pith forms the central part of the tuber. It is broadest near the middle. In its course it gives off at intervals branches which in the form of laterally compressed hollow cylinders communicate with the lateral branches of the tuber—the eyes. The relative lengths of these branches and the angle which they form with the pith varies greatly, but is, nevertheless, closely related to the phyllotaxy and the steepness of the spiral formed by the eyes. The general appearance and the size of the pith and its branches also varies, but is more or less constant for a given variety. The pith either terminates abruptly in the region of the apical bud, or it branches out some distance below the apex, each branch communicating with one of the eyes of the apical eye cluster. In either case, however, the terminal bud forms a broad connection with the pith tissue—a fact which seems to be related to the time of renewal of growth.

The cortex forms a narrow band of tissue limited internally by an almost uninterrupted circle of phloem groups, externally by the periderm. In the young stolon, as has been previously noted, the cortex occupied a large area compared with the organ as a whole, but in the later development of the tuber the cortex adds but little new tissue, and hardly more than doubles the number of rows of cells in the radial extent.

The mature cells of the cortex, like those of the pith, are polyhedral, with a median diameter of about 180 microns. The periclinal walls are larger, because of the great peripheral growth of the organ. Compared

[2] Reference is made by number (italic) to "Literature cited," pp. 834-835.

to the cells of the pith, however, the cortex shows a greater degree of specialization. Some of the cells, especially in the region of the eyes, have greatly thickened walls, forming typical stone cells or sclereids. Not all varieties develop these stone cells. The peripheral cortical cells either abut abruptly on the periderm or form a transition zone three to eight cells wide. The cells in this transition region are smaller than the typical cortical cells, more elongated, and nearly rectangular. They are almost devoid of starch, but contain proteins, especially in the form of cubical crystals, and, in the case of colored varieties, also the pigment.

The epidermis of the tuber primordium has only a temporary existence. Anticlinal walls early appear in certain of the epidermal cells; later periclinal walls are also added. Simultaneously with the division of the cells of the epidermis, division walls also appear in the subepidermal layer. Soon a regular cork cambium becomes distinct, and cell division in this layer continues until a layer of tissue from 6 to 15 cells wide is produced, which assumes the protective function of the epidermis. The cork cambium, or phellogen, consists of a single layer of cells which divide tangentially and which constitute the inner row of daughter cells produced by the first division of the cells of the hypodermal layer. While most of the periderm arises from the phellogen derived from the hypodermis, the young tubers contain in addition a superficial periderm derived from the epidermis.

In the young tuber primordium the first periderm cells naturally appear in close proximity to the stem end, but soon the periderm extends over the entire tuber, and by the time the latter has reached only the size of a pea the characteristic number of rows of periderm cells has developed. The cork cambium remains active throughout the season, forming new cells to replace those which are sloughed off at the surface as the tuber expands. Loss and gain are thereby balanced, and the tuber shows through the various stages of its existence the normal thickness of skin, unless seasonal changes constitute a factor which changes the normal development.

The individual periderm cell is approximately brick shaped; its walls remain thin and later become suberized. The cells abut on each other, almost invariably without intercellular spaces. Small simple perforations are rarely observed (Pl. 10, K). The mature periderm cell is devoid of content. The younger cells contain small granules of tannin and the remains of the nucleus. Occasionally starch grains are found, but the latter are small, round, and do not show the striation characteristic of the large grains. In colored varieties the periderm cells contain the pigment, which, as has been previously noted, is also found in the peripheral cortical cells.

Simultaneously with the formation of the periderm the cells underneath the stomates, which are found widely scattered over the surface of the tuber primordium, begin to divide actively, producing a mass of loose tissue of roundish cells. Under favorable conditions these cells break through the epidermis and proliferate, becoming visible even to the naked eye as small white dots. Since the periderm is quite impervious to gases, this new structure—the lenticel (Pl. 8, E)—performs the function of aeration. This is facilitated by the shape of the cells and by the course of the intercellular spaces which run in radial direction.

Under certain abnormal environmental conditions it may happen that the periderm departs from the normal development. Such a condition was observed in Bliss Triumph potatoes grown in Ithaca, N. Y. The

tubers were, on the whole, normally developed; an examination of the periderm, however, showed that it differed from the normal in structure. The outer rows of cells were greatly hypertrophied (Pl. 8, E, D). The enlarged cells were found in all parts of the tuber, even covering the lenticels. Since other varieties grown under identical conditions showed a normal periderm, no satisfactory explanation was forthcoming for this abnormal formation.

If potatoes are cut and left in suitable surroundings, the surface of the cut becomes covered by a new "skin"—a wound periderm. The new cells appear in certain places and spread in all directions; the walls are laid down in a tangential plane. The cells resemble the normal periderm cells and, like the latter, perform the function of resisting evaporation and fungus attack. In this capacity the cells of the wound periderm appear to be even more effective than the normal periderm cells. Conditions which affect wound periderm formation have been the object of a great deal of investigation. The latest contribution, together with a review of the older literature, is by Edson and Shapovalov (*4*).

As the stolon enlarges to form the tuber, the ring of bundles begins to lose its definite arrangement in groups, and, since the development of parenchyma within the bundles tends to split up the separate groups still further, the entire vascular tissue becomes greatly spread out to such an extent that the vascular ring of the mature tuber can be compared only by analogy with the original one.

Cambium activity becomes evident even before the stolon tip enlarges to form the tuber, but the number of secondary elements thus added is conspicuously small, and only under favorable conditions does it attain the proportions shown in Figure 6. The secondary xylem cells are large, mostly porous, vessels with side walls heavily pitted (Pl. 2, E). The length of the elements varies greatly wherever the cells have been deflected from their normal course. Separating the radial rows of vessels are parenchyma cells homologous with the rays of the aerial stem. These cells rarely contain starch and their walls remain cellulose. The new phloem elements differ only in size from the cells of the primary tissue, and, like the former, they occur in small groups. The parenchyma cells surrounding these phloem groups are smaller than the normal storage cells, more elongated, and contain only fine-grained starch.

In their entirety the tissues so far discussed—namely, cortex, pith, and vascular ring—form only a small part of the tuber. By far the larger bulk of tuber tissue lying between cortex and pith and divided into two unequal parts by the narrow vascular ring consists of large polyhedral parenchyma cells in which small islets of phloem are embedded. What is this tissue morphologically? An inquiry into the ontogeny of the tuber should assist in arriving at a satisfactory solution (fig. 3).

The first change concomitant with tuber formation is an enlargement of the stolon in radial direction. This is brought about by successive cell division in the region of the pith. As a result of this localized growth, the vascular elements become deflected from their normally vertical course and are forced to continue in a more or less oblique direction. Simultaneous with division in the pith cells are changes in the peripheral cortex. To meet the dilation of the center of the stolon, the cells begin to divide radially whenever tangential stretching is unable to accommodate the constantly widening circumference of the organ. At the same time the cortical cells become filled with starch and occasionally dark cells of crystal oxalate appear among the white starch cells.

Soon after cell division in the pith is at its height and the above-described changes are going on in the cortex, the pericycle and the *Markkrone* become the regions of greatest growth. The cells of the pericycle surrounding the primary groups of the outer phloem begin to divide and to enlarge rapidly, and as new cells are formed the older ones become filled with starch and soon become continuous with the cortex; in fact, they appear like wedges of the latter thrust into the zone of phloem tissue. However, their appearance, while the continuity of the endodermis still exists, permits of no other interpretation than that these cells are procambial in origin. Gradually, as the result of continuous cell increase in this tissue region, the cells of the endodermis fail to keep pace with the

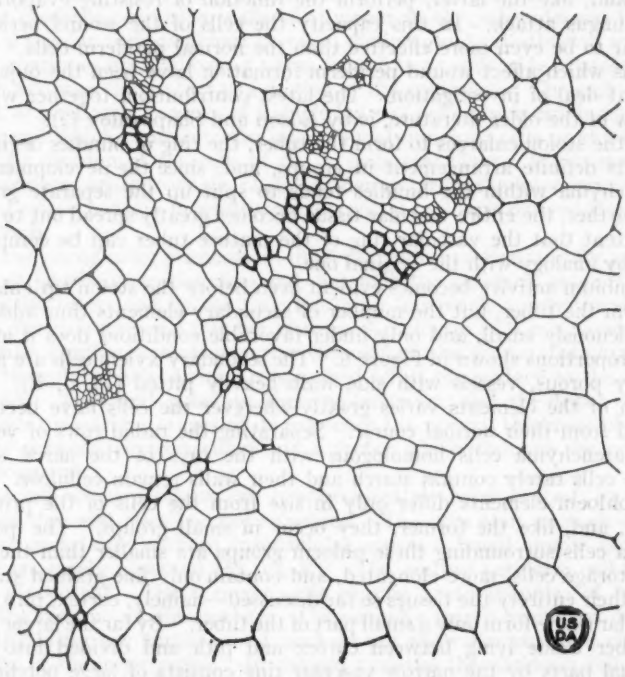

FIG. 2.—Cross section of vascular tissue of mature tuber. ×73. (Drawing is a copy of photomicrograph.)

increasing circumference of the vascular tissue. Gaps appear here and there, gradually widening till finally all traces of this cell layer are lost in the starch-filled parenchyma, and only the outer circle of phloem groups indicates its former position. As a result of the activity of the pericyclic cells, the phloem groups become spread out; the individual strands become more widely separated from each other. New groups are formed as the tuber enlarges, and, grouped in larger or smaller constellations, they appear to the naked eye as dark dots surrounded by a halo of white starch cells. Detailed microscopic study shows the smaller groups to be made up of aggregations of the elements found in the ordinary primary phloem groups; i. e., a few sieve tubes with their companion cells and a number of conducting parenchyma elements. The cells surrounding

the phloem groups are smaller and more elongated than the storage cells and contain only small amounts of fine transitory starch.

To recapitulate: The tissue outside the vascular ring is composed of a narrow cortex analogous to the original cortex of the stolon and a much wider area of storage parenchyma containing numerous groups of phloem. This tissue is procambial in origin, although very much changed in its later development on account of the rôle which it is destined to play in the function of the tuber as an organ for storage.

Changes such as take place in the pericycle also proceed in the *Markkrone*. Rapid and continuous cell division causes here also a spreading

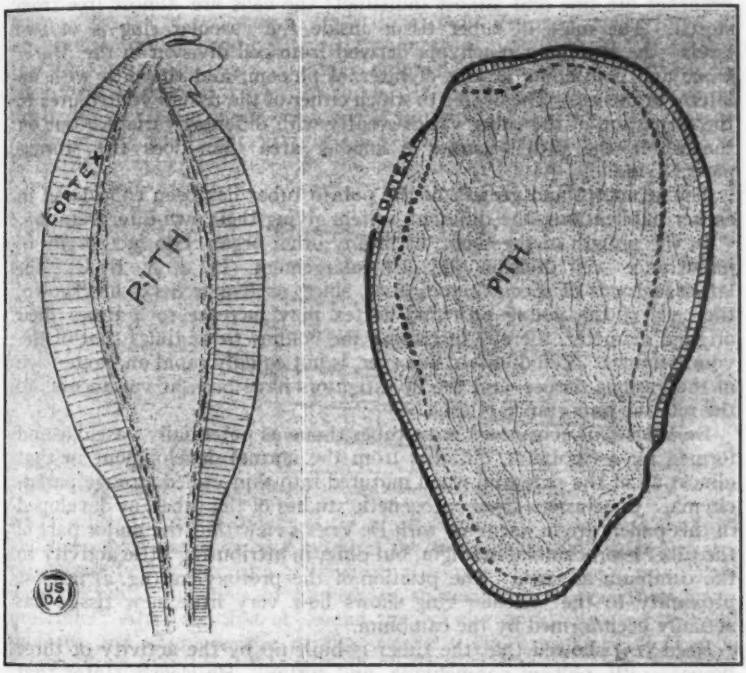

FIG. 3.—Diagrammatic drawings of radial section of stolen tip (×9) and mature tuber (normal size) to show homology of tissues.

and deflection of the phloem groups centripetally. The activity continues till the tuber is mature. A certain amount of cell division also takes place in the region of the primary xylem whereby the protoxylem elements become more and more separated from the metaxylem, as seen in Figure 2. The band of tissue resulting from such growth, however, is inconspicuous.

In the mature tuber the vascular ring approaches the periphery in the region opposite the eyes, branching out in the buds and bud scales of the latter. The central pith is continuous with the eyes, as can easily be seen when a tuber only a few millimeters in diameter is examined. When the tuber enlarges and the distance between the peripheral eyes

and the center of the tuber naturally widens, the pith retains its connection with the eyes and its buds. Thus we see in the mature tuber branches radiating from the pith toward the periphery of the tuber (Pl. 1, A. B). In tangential section these branches appear as compressed hollow cylinders inclosing groups of phloem tissue. These pith branches, however, are not always symmetrical, since, on account of irregular growth activity, groups of phloem cells become more and more subdivided and spread out, causing a temporary or permanent breaking up and subdivision of these pith branches—a condition which becomes more conspicuous toward the periphery. In certain varieties the pith branches are large and uniform; in others, notably the Triumph, the branches are fine and almost indistinct; the cells are almost free from starch. The mass of tuber tissue inside the vascular ring is of two kinds—the storage parenchyma derived from cell division in the *Markkrone* and containing groups of internal phloem, and the pith with its lateral branches. The degree to which either of the tissues contributes to the formation of the tuber varies greatly with different varieties, but on the whole the pith occupies a smaller area than does the storage parenchyma.

The structure and growth of the potato tuber has been considered in earlier publications, the different writers giving their own interpretation.

In the growth of the tuber, as in any other organ, two factors are of importance—cell division and cell enlargement (Pl. 4, A, B, C). The latter factor is of special significance, since, as Esmarch (*5*) has shown, the cells of the young pith and cortex may increase to 4 times their original diameter, thereby increasing the volume to 64 times that of the young tissues. Cell division, however, is not equally rapid and extensive in the various tissues, and the investigators have been at variance as to the relative importance of each.

De Vries (*20*) considered most tuber tissue as potentially vascular and formed by a cambium, differing from the normal development in that almost all of the potential wood matured into thin-walled storage parenchyma. Conclusions from ontogenetic studies of the tuber, as developed in this paper, are in harmony with De Vries's view that the major part of the tuber is procambial in origin, but differ in attributing little activity to the cambium as such. The position of the protoxylem (fig. 2) in close proximity to the vascular ring shows how very little new tissue has actually been formed by the cambium.

Reed (*15*) showed that the tuber is built up by the activity of three tissues—pith, phloem parenchyma, and cortex. He clearly states that the cells between the internal phloem and the protoxylem begin to divide very early, causing a spreading out of the phloem groups. While this is in agreement with the writer's observations, it is difficult to assume that numerous cell divisions occur also in the parenchyma of the phloem groups and that through this activity a large amount of tissue is added to the growing tuber. It appears rather that the phloem parenchyma must be considered an integral part of the phloem groups and that its cells at maturity do not become storage parenchyma. The cell divisions which contribute to the increased size of the tuber must be sought in other tissues, and, as the writer has shown, most likely among the undifferentiated cells of the procambium.

Esmarch (*5*) regards the enlargement of the pith as the chief factor in tuber growth. He considers the internal phloem groups to be of

independent origin and in no way related to the vascular cylinder. However, the ontogeny of the vascular tissue, especially of the isolated groups of phloem and their subsequent development, excludes such a theory.

THE POTATO EYE

The potato eye is a leaf scar with its subtended axil, which contains a suppressed lateral bud and undeveloped internodes. The eyes in their entirety show a definite arrangement in the form of a spiral (fig. 4), the direction of propagation of which is, according to the variety and the individual, either right or left. The arrangement of the eyes is thirteen ranked, since the fourteenth eye is over the first after five turns of the spiral. Each eye contains at least three buds arranged in the form of an obtuse triangle and protected by more or less conspicuous scales (fig. 5). Often, however, a larger number of buds are present which then form a secondary spiral to the left if the main spiral of the eyes is dextrorse, or conversely if the latter is sinistrorse.

The eyes of the tuber vary greatly in size and form, and the difference is further emphasized through the influence of environmental factors

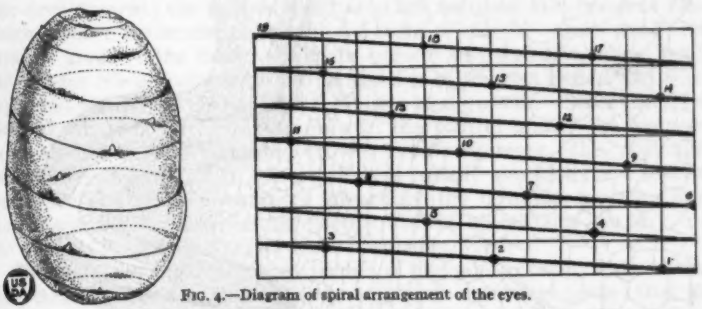

FIG. 4.—Diagram of spiral arrangement of the eyes.

which affect the development of the plant in general. There are shallow and deep-eyed varieties, and between the two extremes all intermediate types may be found. In a given tuber the lowest basal eye is small and inconspicuous; the eyes in immediate succession are larger and may protrude. After the first or second turn of the spiral the eyes become uniform and characteristic of the variety. The apical eye cluster is commonly not in direct line with the main axis, but excentric; the eyes composing it are small and contain fewer buds than those of the body of the tuber. There is, furthermore, a variation in the depth of these eyes. Some are very protuberant, but most are quite shallow.

A median longitudinal section of a stolon tip prior to tuberization shows the apical growing point with its protective scales and a number of buds developing in the axils of scaly leaves (Pl. 3). As the tuber enlarges, new buds are constantly differentiated from the growing apex, while the older ones gradually develop to maturity. In this growth and differentiation the scales, once very prominent, show a decided lag in development, and finally atrophy, leaving a mere scar, the "brow" of the mature eye. The size and form of the brow is correlated with the depth of the eyes. As a rule, the deeper the eye the more prominent the brow.

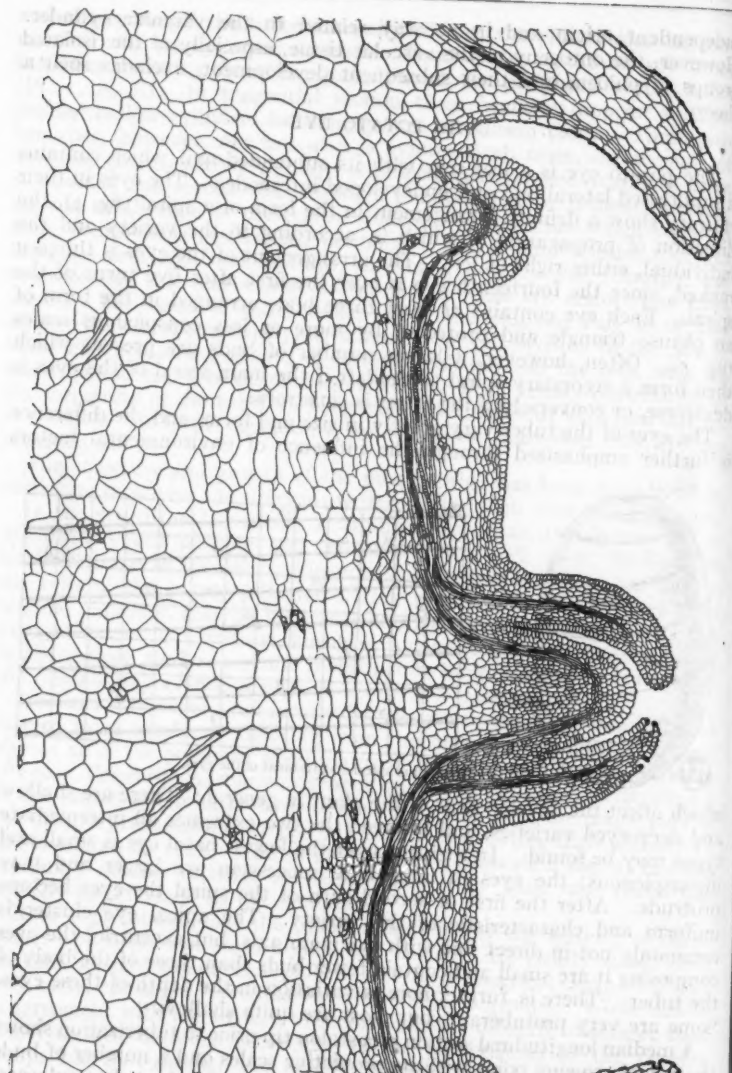

FIG. 5.—Drawing illustrating structure of potato buds and surrounding tissue.

In structure and tissue differentiation the resting bud resembles a stolon tip. The apical region, well protected by overlapping scales, is composed of a mass of cells rich in protoplasm. The walls are thin and of cellulose except for the outer wall of the epidermis, which is covered by a thin cuticle. Since the latter is devoid of a cellulose basis, as shown for other plants by Priestley, parasitic fungi which secrete cytolytic enzymes can penetrate the cuticle only by force of hydrostatic pressure. Where the bud merges into the tuber, differentiation into the characteristic tissue region takes place and the vascular tissue of the bud and bud scales joins the vascular ring of the tuber, which in the region of the eyes closely approaches the periphery. Like the vascular ring, the pith retains its connection with the lateral members of the tuber in that branches of the pith continue directly into the eyes. Since all the eyes thus maintain a connection with the central pith, sections through any part of the tuber show a system of such connecting strands of pith radiating from the center toward the periphery. The size and shape of these pith connections are similar in tubers of a given variety, and may, as will be shown later, serve as a group character.

With the termination of the growing season, the tuber has completed its development; the bulk of the tissue has matured and becomes filled with reserve materials, chiefly in the form of starch. Only small, restricted areas of the tuber, the buds, possess meristematic tissue, ready to resume life activity with the completion of the rest period and when external conditions permit of cell division and growth. Since the tuber is a shoot with indeterminate growth, the central bud is the youngest and should normally resume growth first. However, the prolonged state of dormancy which affects all eyes equally should have a leveling influence and remove whatever advantage the terminal eye may have possessed. Yet the earlier development of the apical buds is a fact, and to explain it various theories have been considered.

Franz (*6*), in his anatomical studies of the potato tuber, noticed that the terminal eye was shallow and not buried in cortical tissue; that the cells in the vicinity of the bud contained more water and were richer than elsewhere in protein; that the pith formed a direct and broad connection with the apical bud; and since the latter was also ontogenetically the youngest he concluded that the morphological and chemical differences sufficed to account for the earlier development of the terminal eye.

While the evidence brought forward by Franz may not be contested, it must be remembered, nevertheless, that the vascular tissue of the tuber, no matter whether it connects to lateral or terminal buds, is at best only weakly developed and that the repressive effect of a thick cortical tissue appears nowhere in evidence. The direct and more massive pith connection and the broader point of contact which the terminal eye forms with the vascular tissue are undoubtedly advantages which the lateral eyes lack. This fact is emphasized by the results of some experiments of Miss Fernald,[3] who found a rather definite correlation between the osmotic concentration of the tissues around the eyes and the tendency for these eyes to sprout. While Miss Fernald's results may explain why the basal eyes are inhibited, they in no way explain why the apical eyes get the earlier start. The morphological advantages which the apical eye possesses over the basal ones, as shown above, seem to offer the most reasonable explanation for the observed dominance.

[3] FERNALD, Evelyn I. THE INHIBITION OF BUD DEVELOPMENT AS CORRELATED WITH OSMOTIC CONCENTRATION. (Title.) *In* Amer. Assoc. Adv. Sci. Program 76th Meeting, p. 94, 1922.

CYTOLOGICAL STUDIES

NUCLEUS AND MITOTIC DIVISION.—Neméc (*11*) studied the nucleus and division stages in the cells of the root, the stem apex, and wound periderm. He found noticeable differences in the formation of the achromatic figure in the cells of the wound periderm. In the normal division there accumulates around the nucleus at the beginning of prophase a fine granular substance, in which, at a later stage, two periplasts differentiate at the poles. As these periplasts enlarge, delicate fibers begin to develop from the apex and grow out toward the equator, where they unite to form the spindle. When the spindle is formed, the nuclear membrane disappears and the chromatin, which during prophase formed a peripherally disposed thread, differentiates into chromosomes which, after undergoing longitudinal cleavage, arrange themselves at the equator. The chromosomes form short thick rods; they number at least 36. The nucleolus contains a single central or several vacuoles in which small, cyanophyllous granules are precipitated in fixed material. The nucleolus disappears with the formation of the equatorial plate, although its residue sometimes persists during metakinesis. Often they appear again, in the vicinity of the poles, at the end of metakinesis. In the cells of the wound periderm the development of the achromatic figure differs primarily in that the spindle fibers are not developed from a hyaline periplast, but grow directly from the surface of the nucleus and parallel to the division axis. The nuclei of the cells of the wound periderm are at least 30 per cent larger than those of the normal cells, and the chromosomes may reach as high a number as 70.

Since we possess in the work of Némec an excellent account of the nuclear phenomena in the potato, it will suffice here to illustrate the principal division figures (Pl. 5) and proceed to a consideration of the types and structure of the nuclei in the various tissues, notably the phloem. In the apical meristem of the young stolon all resting nuclei are spherical (Pl. 5, A, B, and Pl. 7, C), with a mean diameter of about 12 microns. Each nucleus is composed of a large nucleolus surrounded by a hyaline sphere and a homogeneous nuclear substance in which chromatin granules of various sizes are embedded. The same type of nucleus, although often of larger dimensions, is found in all unspecialized parenchyma cells of the tuber. In the cells of the procambium and their derivatives the nuclei show a great variety of form and structure. They are often elongated with ends either blunt or pointed (Pl. 5, C–G and Pl. 7, A, B). Often they are rod-shaped, reaching a length of 30 microns or more. Normally there is one nucleolus to a nucleus; in the large and elongated forms, however, two or even three nucleoli may be present (Pl. 5, G).

The nucleus of the young sieve tube is large and fusiform. There is, as a rule, one nucleolus; occasionally one or two secondary nucleoli are found. As the sieve tube matures, the dense protoplasmic content, so conspicuous in the young element, becomes limited to a peripheral layer, while the nucleus itself gradually disintegrates. In the mature sieve tube, where the sieve plate and slime plug are easily recognized, a nucleus is no longer present. In a few instances the nucleus has been seen to persist in a more or less degenerate form even in the mature element.

The protoplasm of the sieve tube initial is granular and homogeneous, like that of other meristematic cells. At an early period, however,

certain structures differentiate out, which, because of their characteristic appearance, have recently been the cause of some misconception as to their nature and function. This peculiar type of sieve tube inclusion was first observed by Nelson (*10*) in the potato and other plants. The bodies were described as straight or twisted rods with pointed ends and occasionally bearing an attenuate process at one or both ends. The true value of the discovery was temporarily marred by an attempt to identify these bodies with certain flagellates and ascribing to them a probable rôle in the production of the mosaic disease. However, although these bodies were wrongly identified and their supposed connection with the mosaic disease was further invalidated by the fact of their occurence in healthy as well as in diseased plants, their discovery is not without interest. An insight into their origin and nature was much to be desired, and some attention has been given to them in this morphological study of the potato tuber.

The bodies first become noticeable as light staining, looped or undulating bands along the inner margin of the cell or, more commonly, adhering to one side (Pl. 7, G). Often the bands are short, pointed and somewhat spirally twisted (Pl. 7, E, H; Pl. 6, C, B, E) abutting on the nucleus of the cell (Pl. 7, G) or lying next the end wall (Pl. 6, A, B, E). The bodies exhibit an extreme polymorphism, which is very pronounced within a single element (Pl. 7, F). Once formed they appear to retain their shape and change only in staining quality. They show at an early stage a strong affinity for acid fuchsin (Pl. 1, D) and safranin. When carefully stained with Haidenhains haematoxylin, they present distinctly a dark staining outer shell and a lighter interior (Pl. 6, D), in which are embedded dark granules. Old bodies may, however, stain uniformly black.

These structures usually originate in the cytoplasm of the sieve tube, yet in some cases their nuclear origin appears indisputable. They are abundant in the sieve tubes of the growing apex, but are also found in more mature tissue where new phloem groups are developing; they are typically a constituent of the young element, since they are not observed in cells with a well-developed sieve plate. Their elective power for acid fuchsin and their positive reaction in certain microchemical tests (Biuret, Millon) show them to be protein in nature. They are not typical protein crystals, but rather bodies homologous to those described by Strasburger (*18, p. 196, Pl. 3*) and Mrazek (*9*) for the Papillionaceae. They are undoubtedly concerned in the metabolism of the sieve tube, but their real significance leaves room for much speculation for which only comparative studies on an extensive scale can give a proper basis.

CHEMICAL CONSTITUTION OF THE TUBER

ORGANIC STORAGE PRODUCTS.—The most important reserve material of the tuber is starch, which is deposited soon after the stolon tip swells to form the tuber. The first starch is formed in the cells near the vascular tissue, commonly in the form of fine round grains. Somewhat later starch becomes noticeable in the cells of the cortex, and finally in the pith. The typical grains are ovate, about 40 to 100 microns long. In cross section the grains are spherical. While the small starch grains are more or less homogeneous, the large oval grains have an excentric hilum and show distinct striation. Compound starch grains are occasionally

observed in which each component has a hilum and striations of the nature found in the large individual grains.

Nitrogenous substances in various forms, as constituents of the protoplasm or dissolved in the cell sap, are found most abundantly in the cambium, the phellogen, and in the region of the buds; also in the form of crystals in the peripheral cells of the cortex. The protein crystals appear very early and can be found even in the tuber primordium. They increase rapidly in number and size as the tuber enlarges, so that in the mature tuber more than one may be observed in a cell. The crystals are always cubical in form, but their size varies greatly. The larger and more typical crystals have a diameter of 12 microns (Pl. 1, C). Upon germination the cleavage products of the protein crystals form, according to Sorauer, the first food for the developing buds.

A small amount of fat is found in the periderm cells and in the peripheral cortex. Inulin is absent, but organic acids—citric, succinic—are present, lending the tuber an acid flavor which is apparent even without analysis. Sugar is not found in the mature tuber, but becomes abundant upon renewal of growth.

CRYSTALS.—Besides the typical protein crystals described above, two other types are commonly observed—calcium oxalate, in the form of crystal sand, found in special cells and observed first in the cortex of the young stolon. In the older tuber those cells often occur in vertical rows. The crystal sand consists chiefly of small crystals of calcium oxalate in the form of octahedrons and their derivatives. Together with the calcium oxalate there is always a protein matrix which remains after the crystals have been dissolved by acids. The crystal-bearing cells increase in number and appear not only in the cortex but also in the pith. In the mature tuber they disappear, but become evident again in large numbers in the region of the buds in the germinating tuber. In the peripheral cortical cells there are found occasionally crystals of basic calcium phosphate. These form four-sized prisms with pointed ends; however, they appear rarely in pure crystal form and often contain coloring matter. In time of their appearance the phosphate crystals are later than calcium oxalate. Sorauer considers calcium oxalate crystals a decomposition product of the carbohydrates, and the phosphate crystals a residue of protein metabolism.

SOLANIN.—When a section through an eye is treated with alcoholic phloroglucinol, it will be observed that the region of the buds stains a deep orange. The color is most intense in the epidermis and in the parenchymatous cells inside the vascular cylinder. Similarly, when a section is treated with concentrated sulphuric acid or ammonium vanadate with sulphuric acid (1 part of ammonium vanadate is dissolved in 1,000 parts of sulphuric acid, prepared by mixing 98 parts of sulphuric acid with 36 parts of water), the following color reaction is observed: Yellow, red-yellow, brown-red, dark red, violet, fading out. According to von Brehmer (*2*), who studied the distribution and migration of solanin in the potato, there is only one color change, from yellow-red to violet; all other colors merely indicate different concentrations of solanitn. Von Brehmer found in his studies that solanin can not be extracted with water and is not held in combination by acids, and suggested that it is held in solution by colloidal proteins. Upon germination the solanin content diminishes in the peripheral cortex and becomes evident in the elongating shoot. Solanin is a glucoside which upon hydrolysis gives solanindin, galactose, rhamnose, and, as an intermediate product, a complex sugar.

It dissolves easily in hot alcohol, slightly in benzol, cold alcohol, and ether. Solanin is found in large quantities in the young tuber; in the mature tuber only in the region of the buds. Upon renewal of growth it begins to accumulate in larger quantities, and as it gradually disappears from the base of the tuber it becomes more evident in the shoot. The distribution and formation of solanin suggest that it is important in the metabolism of the growing plant. If germination is held back in the spring, solanin may accumulate in such quantities in the tuber, as von Brehmer has shown, as to be poisonous to man.

TANNIN.—One of the most conspicuous and interesting forms of cell inclusions is tannin. In the growing tip it occurs in two forms, as fine granules, notably in the older periderm cells, and in the form of spherical vesicles in the neighborhood of the buds (Pl. 8, A, C, and colored Pl. 1, C). The vesicles occur abundantly in the germinating tuber. They color blue with iron salts and black with osmic acid, and of themselves may be brown or blue. Their content is either a fine granular substance or a homogeneous brown fluid. The larger vesicles, which sometimes fill the entire cell, often contain a number of small globules within them. Sometimes the vesicles are of a lighter color, or even colorless; sometimes they form mere refractive drops. Sorauer (17), who made an extensive study of the nature and distribution of these bodies, observed that the white tubers contained mostly brown vesicles and that in colored varieties a mixture of brown and blue vesicles was found.

STRUCTURE OF THE STOLON WITH SPECIAL REFERENCE TO FOOD CONDUCTION

In the structure of the mature stolon the epidermis and the vascular tissue merit special consideration. In the tuber, as we have seen, the epidermis is replaced by a many-layered periderm, while in the stolon the epidermis is retained, but its outer walls and cover hairs become lignified. In the development of the vascular tissue strength has been sacrificed for efficiency in conduction. The xylem forms a continuous ring, composed mostly of large porous vessels. The area occupied by the outer and inner phloem is relatively greater than that in the stem. Sieve tubes are numerous and of a large diameter. The sieve tubes of the secondary phloem are especially large and measure as much as 20 microns in cross section.

An average-sized stolon bearing a medium-sized tuber had the following tissue areas (fig. 6 and Table I):

TABLE I.—*Relative areas occupied by the different tissues of the cross section of a mature stolon.*

Stolon tissues measured.	Area.	Per cent of total.
	Sq. mm.	
Cross sectional area of stolon	2.130	100.0
Cross sectional area of outer phloem	.277	24.2
Cross sectional area of inner phloem	.239	
Cross sectional area of xylem	.174	8.2
Cross sectional area of pith	.350	16.3
Cross sectional area of cortex	1.090	51.2

Dixon (*3*) obtained similar figures for the phloem area of potato stolons. (A stolon 1.6 mm. in diameter had a cross sectional area of the phloem of 0.42 sq. mm.) From experimental evidence and theoretical considerations, Dixon concluded that organic substances could not pass through the phloem tissue in such amounts as must pass during the active growth of the tuber and that it is the xylem which conducts most of the food. However, in the stolon the xylem does not exceed the phloem area, or is, at best, only twice the area of the sieve tubes proper. In the tuber the xylem is greatly reduced, while the phloem is correspondingly increased. For the stolon it may be granted that xylem and phloem share equally in the transport of organic substances; in the tuber, however, the phloem and the extensively pitted storage parenchyma are probably the channels of translocation.

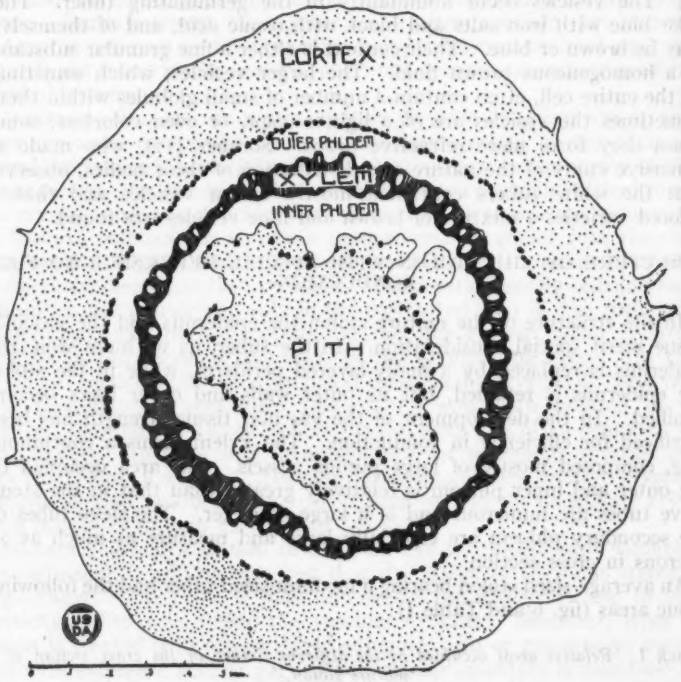

FIG. 6.—Diagrammatic drawing of cross section of mature stolon, giving the relative areas occupied by the different tissues.

COMPARATIVE STUDIES

Differences in the external morphology of the tuber, such as shape, color, type of eyes, have been used in the classification of potato varieties. It was found, however, that climatic and soil conditions greatly impaired the diagnostic value of these characters, and therefore it became necessary to consider the plant as a whole in the classification scheme. An anatomy of the potato tuber would be incomplete without at least an attempt at a study of such internal characteristics as might aid in the task of grouping potato varieties or establishing their relationship. Yet, if such a study is to be fruitful, the effect of environment, so potent in

shaping the external form of the tuber, must be taken into consideration, not for one, but for a number of generations.

In the present study only one generation of plants grown in different localities (the States of Maine, New York, Pennsylvania, and Colorado) was included. The suggestive results obtained from this preliminary study appear to warrant further investigations along this line.

In this comparative study the following terms, as defined, are used for convenience only, and are not considered as having strict morphological significance:

Pith: The central region of the tuber extending as an axial strand from stem end to apex.

Medulla: Lateral connections which the pith forms with the buds.

Cortex: Tissue between cambium and periderm.

Periderm: The potato skin.

Stone cells: Cortical cells which possess a thick, lignified wall.

PITH AND MEDULLA.—The pith forms the axial core of the tuber. It varies in thickness, attaining its greatest diameter a little below the center. At intervals branches in the form of compressed hollow cylinders leave the pith, take a more or less oblique vertical course, and connect each with one of the eyes. These medullary branches attain various proportions, and in cross section exhibit configurations which are more or less characteristic of the variety. The medullary branches vary in both form and distinctness. To be sure, young growing tubers have a less distinct pith and medulla than mature tubers, especially if the latter have been in storage for a certain time; yet when examined under identical conditions the tubers show differences which are distinct and form a constant feature of the different varieties. In tangential section the medulla branches appear in the form of open or closed horseshoe-shaped areas, and as the radial cut is approached the relation of these areas to the pith and eyes can clearly be seen.

CLASSIFICATION OF VARIETIES

Medulla distinct and large, antler-shaped; pith commonly large.—*Cobbler, Rural* (Pl. 9).

Medulla distinct, either large or small; pith large.—*Ohio, Up-to-date, McCormick* (Pl. 9).

Medulla branches distinct and fine; pith usually small.—*Triumph, Green Mountain, Rose* (Pl. 9).

Medulla branches fine; pith either large or small.—*Michigan, Hebron, Pearl* (Pl. 9).

Medulla branches indistinct; pith varying in size.—*Burbank.*

This classification, based on median cross-section view of tubers, is at best only a tentative one. While the medulla configurations are fairly constant, the pith is very variable, especially in tubers which are off type. Environmental factors appear to be of subordinate importance, since marked local variations are very pronounced; in other words, tubers grown in Maine and New York, for example, show no more variation than those from a given field in either State.

CORTEX.—The cortex forms a narrow band of tissue limited externally by the periderm, internally by the cambium of the vascular ring. In the region of the eyes the cortex becomes narrow and disappears completely as the vascular tissue branches out into the buds of the eye. Aside from the variations in the region of the buds, the width of the cortex throughout the tuber shows a more or less pronounced fluctuation.

These differences may be local or affect an entire side, so that one longitudinal half of the tuber has a broad cortex, the other half a narrow one. External irregularities also tend to affect the width of the cortex; but an external swelling may not always indicate a correspondingly wider cortex; on the contrary, this tissue in such regions is often narrower (Pl. 9).

CLASSIFICATION OF VARIETIES

Wide cortex (8–11 mm.).	Medium cortex (6–7 mm.).	Narrow cortex (3–6 mm.).
Hebron.	Cobbler.	Green Mountain.
Michigan.	Rose.	Triumph.
Early Ohio.	Burbank.	Rural.
McCormick.		Spaulding Rose.
Up-to-date.		
Burbank (seedling).		

The varieties which are most likely to possess an irregular cortex are: McCormick, Hebron, Pearl, and Michigan. In the Burbank group, the varieties have a fairly even cortex, except the Burbank seedling,[4] which shows great irregularities. The latter variety differs further by having a broader cortex than the other members of the group. The Green Mountain and Rural types have as a rule a very regular cortex, though in the Green Mountain the width may sometimes vary. Climatic conditions appear to influence the development of the cortex, inasmuch as rapid growth in the early development of the tuber tends toward the formation of a broader cortex. However, it is safe to assume that a wide or narrow cortex, if constant in a number of specimens, is characteristic of the variety regardless of the external conditions under which the tuber was grown.

STONE CELLS.—When tubers of certain varieties are sectioned through the region of the bud, large lignified cells are noticed. The occurrence of these so-called stone cells is so constant that potato varieties can be divided into two classes—those in which stone cells are present and those in which they are wanting. While the presence of stone cells is a varietal characteristic, there is a great deal of variation in their number. The Rural group is distinguished by developing stone cells very sparingly, and in some varieties, like Heavy Weight, they seem to be absent altogether.

CLASSIFICATION OF VARIETIES

Stone cells present.		Stone cells wanting.
Numerous.	Sparingly developed.	
Peachblow.	Rural No. 1.	Rose.
Burbank (seedling).	Carman I.	Heavy Weight.
Superba Irish.	Green Mountain.	Ohio.
Triumph.	Triumph.	McCormick.
Russet Burbank.	Early Eureka.	Burbank.
Rural.	Pearl.	Hebron.
	Charles Downing.	Russet Rural.
	Carman III.	White Rose.
	Gold Coin.	Spaulding Rose.
		Russet Burbank.
		Cobbler.

[4] Name under which William Stuart is carrying one of the Burbank strains.

STARCH.—The quality of starch is determined by the percentage of large grains it contains. The ability to produce a large percentage of superior grains is, according to Saare (*16*), a variety character, which is reasonably constant. In the breeding of varieties for a definite purpose a knowledge of the starch content of the different varieties might be an important factor which would enable the experimenter to make such crosses as would be most promising in results. Parow (*12*) found in his studies that in the variety Tannenberg the percentage of superior grains was as high as 50, while Weddingen yielded only 11 per cent of high-quality grains. In American varieties a group classification based on the quality of the starch grains could also be easily worked out, especially if there exist such striking differences as in Green Mountain and Cobbler as seen in Plate 2, B, C. Since material for investigation is not available at present, the information to be gained from such a study must be reserved for a future publication.

THE PERIDERM

The periderm of the potato has been the object of frequent investigation, and pictures of its structure may be found in any of the textbooks on botany. A comparative study of the periderm of different varieties, however, began only when differences in the disease resistance of certain varieties focused the attention of the pathologist on this natural and effective barrier to infection—the potato skin.

More than 50 years ago Sorauer made a study of the structure of the periderm of nearly 75 varieties. He made exact measurements of the thickness of the periderm, the number of cell rows, and the size of the individual cells. He noticed that deep planting tended to produce a thinner skin than shallow culture and that fertilization of the soil had the same effect. In his infection experiments with late blight he found that fertilization and deep planting produced a higher per cent of diseased plants and that the red varieties were more susceptible than the white ones. From this he concluded that thickness of the skin is correlated with disease resistance. A few years later, however, it was shown by Ress and Bretschneider that the very thick-skinned varieties were even more readily attacked than the thin-skinned ones, and that thickness of the skin in itself did not insure immunity. In 1908 Kreitz (*7*) extended the work of Sorauer by making extensive investigations on the effect of environmental and soil factors on the structure of the periderm. Kreitz found that the thickness of the periderm of a given variety was not a constant factor, but varied with changes in environmental conditions. In opposition to Sorauer he noticed that dryness tended to produce a thin skin and that the different varieties behaved variously. Thickness of the periderm acquired in a new locality would be retained for a generation or more, even after the tubers were transferred back to their original home. Finally, however, they showed the same kind of periderm as they originally possessed. Application of fertilizers gave varying results in that potassium and nitrogenous fertilizers tended to produce a thin skin, phosphoric acid fertilizers a comparatively thick skin. Since the thickness of the periderm in itself appears to be no factor in disease resistance, Kreitz suggested that the ability to regenerate periderm cells in wounded places would be of importance in preventing infection with bacteria. In this connection the work of Appel (*1*) is of significance. Appel found that in the variety

Daber, which is resistant to bacterial wilt, new cork cells appeared 6 hours after injury, while in the thin-skinned and susceptible variety Apollo, new division walls in the periderm formed only after 36–48 hours had elapsed. Lutman (*8*) emphasizes the fact that it is almost impossible to eliminate the personal equation in determining the thickness of the periderm, since on the same tuber thin and thick areas are commonly found. He noticed that application of fertilizers does not affect the thickness of the skin and that varieties resistant to scab have a thick periderm associated with a type of lenticel which is close textured and partly buried under the skin.

In the present study of the structure of the periderm similar variability which could not be correlated with the variety or the external conditions of growth was encountered. This must be borne in mind in connection with the following descriptions of the periderm, as it appeared to be most typical in the varieties studied.

STRUCTURE OF THE PERIDERM IN POTATO VARIETIES GROUPED ACCORDING TO STUART (*19*)

COBBLER.—Periderm normally thick with somewhat rough or scaly surface. Layer measures 130–156 microns and is composed of 8–9 rows of cells. Variation in thickness is not infrequent, as can be seen in Plate 10, A, B, and Figure 7. The cells vary greatly in size and arrangement. The radial rows are interlocked and the cells narrow, which gives the periderm a compact texture. The average size of most cells is 78 by 13.5 microns. The transition zone is rather wide and gradual (4–5 rows). The cells are elongated, rectangular, or pointed.

TRIUMPH.—Periderm of medium thickness, covered by a thin, rough crust. Layer measures 130 microns and is composed of 6–7 rows of cells. Occasionally as many as 10 rows can be counted, the total width of the layer reaching 156 microns or even more. The periderm cells show a fairly regular arrangement in radial rows; the width of the rows, however, varies considerably. The average cell dimensions are 82 by 13–15.6 microns. The transition zone is wide and the change in cell type is gradual. All transition cells are usually narrow and elongated, but near the cortex they become oblong or even cubical. (Pl. 10 and fig. 8.)

MICHIGAN.—Periderm of medium thickness; surface usually smooth, but in Early Eureka rough and scaly. Thickness of periderm layer 130 microns; average number of rows about 9. The cells are rather uniform and show a distinct arrangement in unbroken radial rows. The average length of the cells is 85 microns. In Early Eureka the cells are wider, measuring on the average 104 microns. The transition to the cortex is gradual. (Pl. 10, D, and fig. 7.)

ROSE.—Periderm of medium thickness and smooth. Layer measures 135 microns and is composed of 6–8 rows of cells. The cells are arranged in even radial rows. The width of the rows varies. There are commonly two types of cells—wide ones, measuring 82 by 13–15.6 microns, and very narrow ones. There is only a narrow transition zone, but transition is nevertheless gradual because the transition cells greatly resemble the periderm cells. (Pl. 10, E, F, and fig. 7.)

EARLY OHIO.—Periderm of medium thickness and covered with a thin crust. Layer measures 117–130 microns and is composed of 6–8 rows. The cells are arranged in even radial rows of elongated, mostly narrow cells. The two types of cells measure 78 by 15.6 and 38 by 14.8–15.6 microns, respectively. There is a broad transition zone; transition itself

is very gradual. The transition cells are oblong or pointed (Pl. 10, G, and fig. 8).

HEBRON.—Periderm normally thick, smooth, or covered with a thin rough crust. Layer measures 143 microns and is composed of 8–9 rows of cells. The rows are usually broad, but the uniformity is broken in

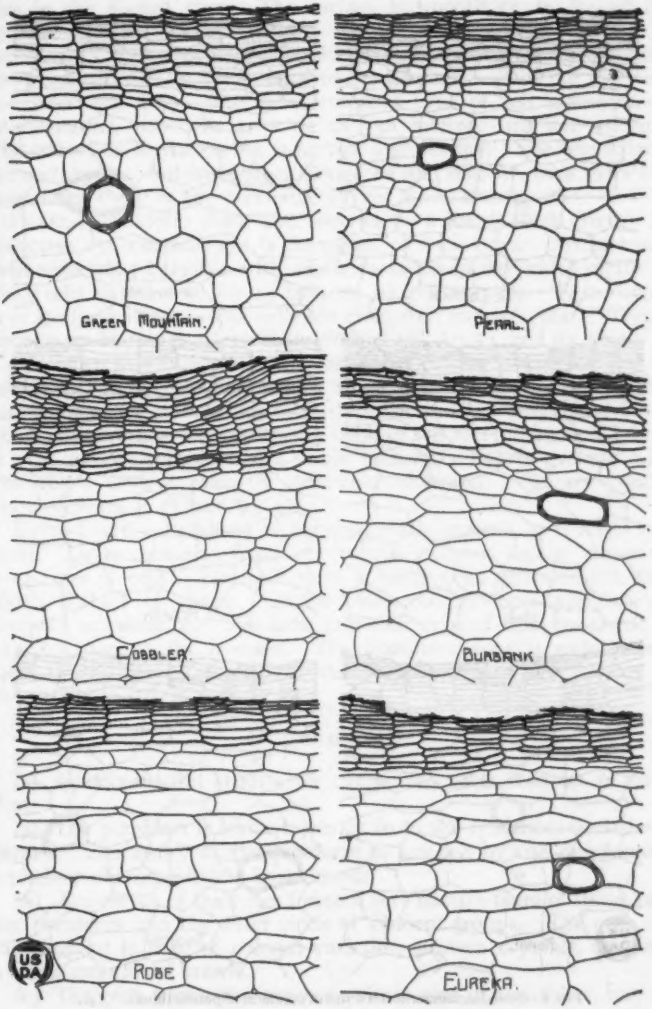

FIG. 7.—Semidiagrammatic drawings of periderm of potato tubers. ×40.

that narrow rows are often interpolated between wide rows. The larger cells measure, 137 by 14.8 microns, on the average. A narrow but gradual transition zone connects the periderm with the cortex. The cells of the transition zone are rectangular; as they approach the cortex they become oblong or polyhedral (Pl. 10, H, and fig. 8).

BURBANK.—Periderm very wide and covered with a rough and jagged crust in the Russet types. Thickness of periderm layer is 156–169 microns; average number of rows 7–11. The cells show a fairly regular arrangement in radial rows; the width of the rows, however, varies greatly. The most common type of cell measures 78 by 18.2 microns.

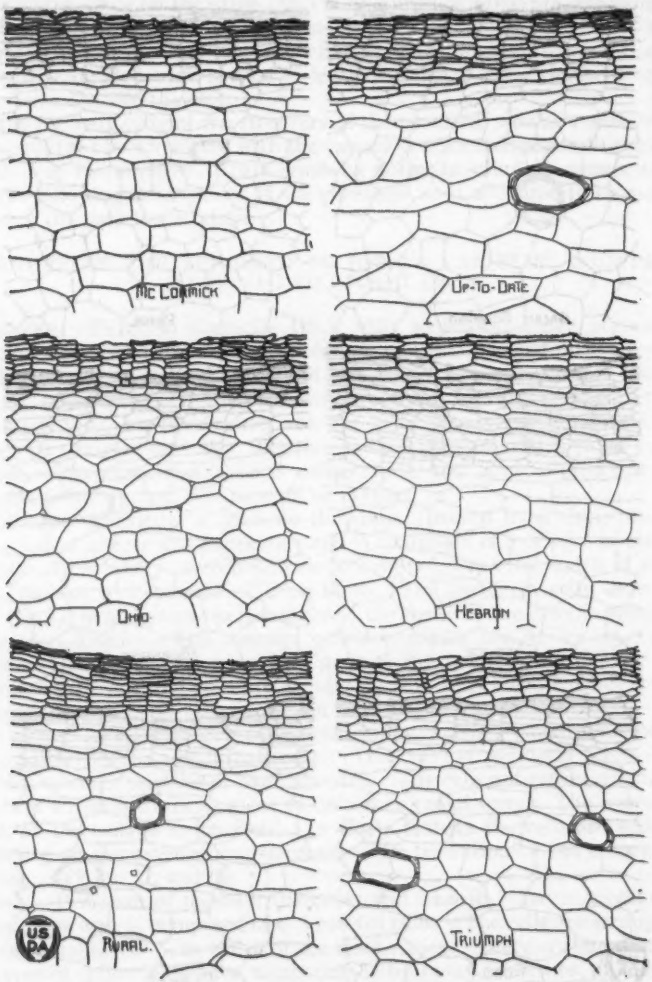

FIG. 8.—Semidiagrammatic drawings of periderm of potato tubers. ×40.

Transition to the cortex is effected by four rows of small periderm-like cells (Pl. 10, I, J, and fig. 7).

GREEN MOUNTAIN.—Periderm thin or of medium thickness, covered with thin rough crust. Layer measures 104–125 microns, occasionally more. Number of rows seven to nine. The cells are uniform and show

distinct arrangement in radial rows. The large cells measure 104 by 14.8 microns, the narrow type 52 by 14.8 microns. Transition is gradual, but the transition cells resemble those of the cortex rather than the periderm (Pl. 10, K, L, and fig. 7).

RURAL.—Periderm thin or of medium thickness, usually more extensive in the Russet type. The surface is smooth or, in Russet Rural, covered with a thick, scaly crust. Layer measures 130 microns, number of rows about seven. Though sometimes uniform, the periderm is more conspicuous by irregularity of the arrangement of the rows and of the individual cells. The most common type of cell measures 91 by 18.2 microns, but cells as wide as 156 microns are not uncommon. The transition to the cortex is narrow and abrupt. The transition cells are rectangular, but sometimes elongated and narrow, thus more closely resembling those of the periderm (Pl. 10, M, N, and fig. 8).

PEARL.—Periderm thick and covered by a rough thick crust. Layer measures 156 microns and is composed of 8–10 rows. Arrangement of rows somewhat irregular with much variation in the width of the rows. Most cells measure 91 by 13 microns on the average. Transition zone is of medium width. The cells are elongated and irregular. Transition appears to be more abrupt than gradual (Pl. 10, O, and fig. 7).

MCCORMICK.—Periderm thick and covered by rough crust. Layer measures 156 microns and is nine rows wide. The cells show a regular arrangement in radial rows of fairly even width. In general the periderm cells are narrow, measuring only 52 by 18.2 microns. Transition is very abrupt; often only a single transition row is present. In the Peachblow several rows of somewhat elongated cells are commonly found (Pl. 10, P, Q, and fig. 8).

UP-TO-DATE.—Periderm of varying width, smooth or covered by thin crust. Layer measures from 104 to 160 microns and is composed of 7–14 rows of cells. The cells show a fairly even arrangement, but the width of the rows varies. On the whole the periderm layer has a very compact appearance. The most common type of cells measures 78 by 15.6 microns, on the average. The transition zone is wide; transition itself is gradual. The cells of the transition zone are usually much elongated (Pl. 10, R, and fig. 8).

SUMMARY

(1) Developmental studies on the potato tuber lead us to conclude that:

(a) The periderm is formed jointly from the epidermis and the hypodermis. Continuity of the periderm is assured by the development of a phellogen arising in the hypodermis.

(b) The cortex of the tuber forms a very narrow band of tissue between the periderm and the outer circle of phloem groups. The cells contain the pigment in case of colored varieties; protein crystals, tannins, and a small amount of starch.

(c) The pith forms the narrow central core of the tuber, but is continuous with the eyes by means of lateral branches. The cells of the pith are poor in starch and have a higher water content than the rest of the tuber issue.

(d) The vascular tissue: The vascular ring as it appears to the naked eye constitutes a narrow band of tissue which contains the xylem and the secondary phloem. The broad bands of storage parenchyma in

which numerous groups of phloem are embedded, though procambial in origin, appears distinct from the vascular ring and is not the result of cambial growth.

(2) The morphological advantage which the apical eye possesses over the basal ones seems to offer the most reasonable explanation for the dominance of the former.

(3) The nucleus of the sieve tubes disappears before the elements fully matured. The peculiar protozoan-like structures observed in have young sieve tubes are of cytoplasmic origin and have only a temporary existence.

(4) In a consideration of the chemical constitution of the tuber, the protein crystals, the tannin vesicles, and the solanin are of special interest. The protein crystals occur abundantly in the peripheral cells of the cortex; the tannin vesicles in the region of the buds, especially at the time of sprouting; the solanin accumulates in large quantities in the region of the buds, where it appears to be of importance in the metabolism of the growing plant. If germination is held back, solanin may accumulate in abnormal quantities.

(5) The phloem of the stolon occupies about 24 per cent of the area of the stolon cross section. Its extensive development, in response to the apparent need for increased food movement, strengthens the view that the phloem is, after all, the most important channel for the translocation of organic substances.

(6) A study of such internal characters as might aid in the task of grouping potato varieties or establishing their relationship indicates that the presence or absence of stone cells is the only definite character which can be used successfully in a classification scheme.

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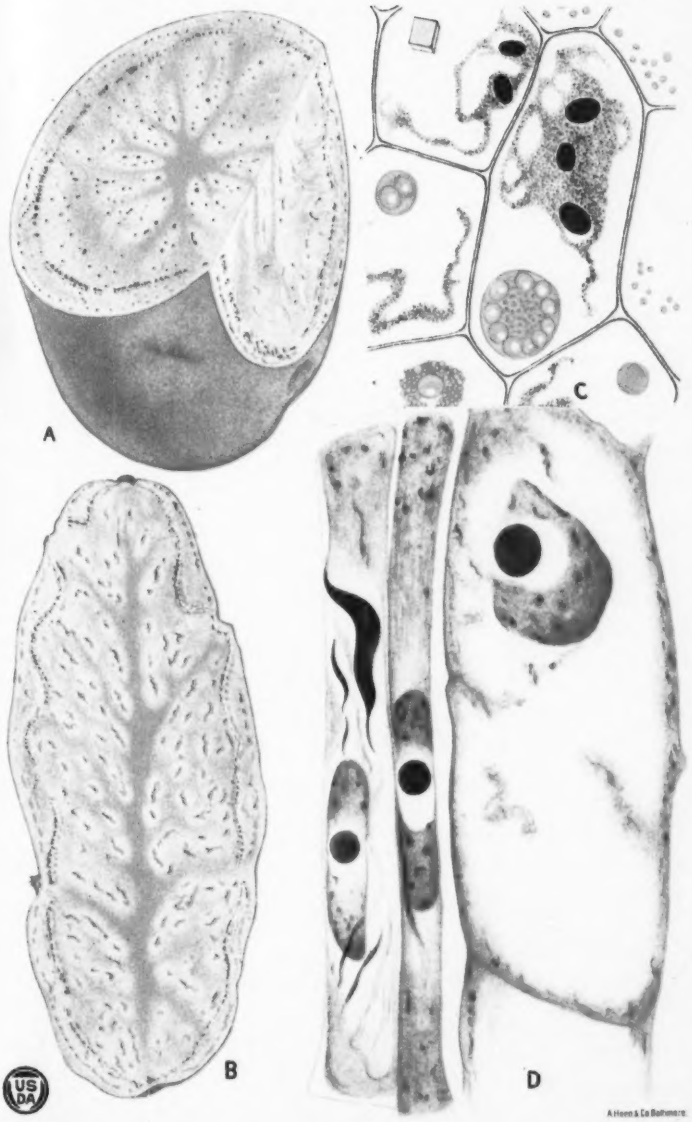
PLATE 1

A.—Potato tuber cut to show transverse and tangential surfaces.

B.—Radial section of tuber to show pith connections to bud. The red line indicates the location of the xylem part of the vascular tissue. Notice that the xylem is confined to a narrow ring, while the phloem is scattered throughout the tuber tissue.

C.—Cortical cells from region just below a bud. The cells contain starch (violet), protein crystals (green) and tannin vesicles (orange yellow).

D.—Protein bodies in sieve tube of young stolon. The cell to the left is a young sieve tube containing a nucleus and spiral bodies (spiral bodies and nucleolus colored red). Adjacent to the sieve tube is a companion cell and next to it a large parenchyma cell. Drawing is a reproduction of a preparation stained with methyl green and acid fuchsin according to Altmann.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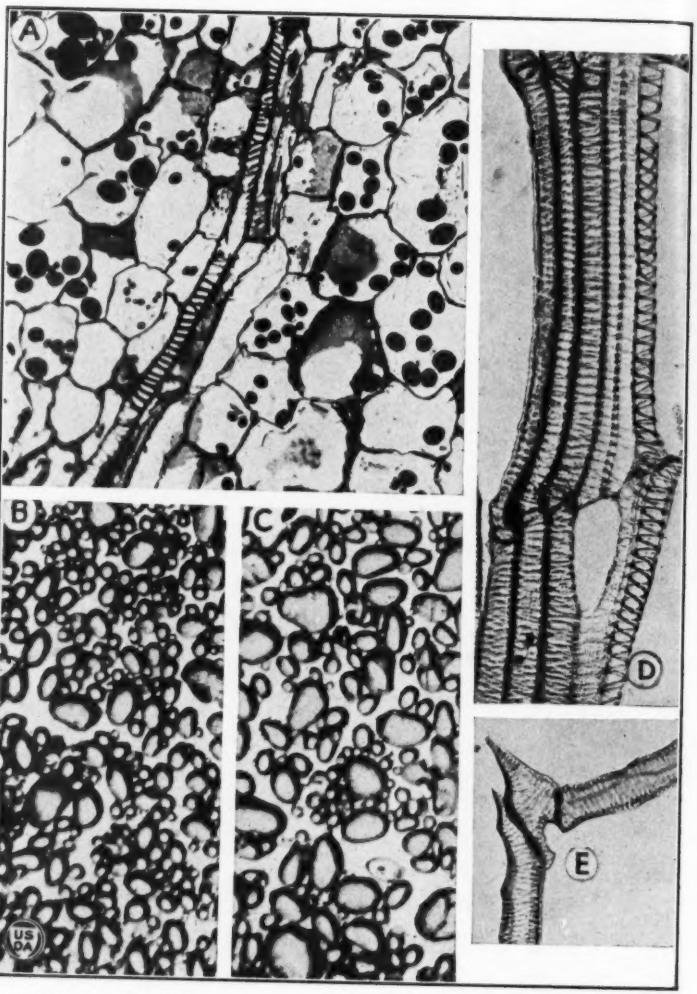
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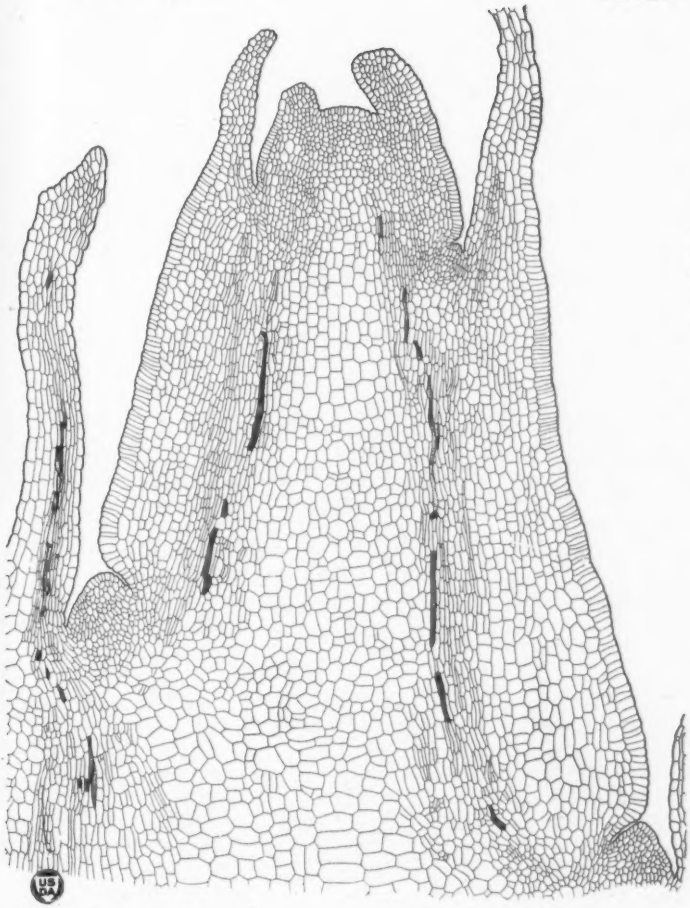
PLATE 2

A.—Longitudinal section through vascular ring of mature tuber. ×129.
B.—Starch grains from the variety Irish Cobbler. ×125.
C.—Starch grains from the variety Green Mountain. ×125.
D.—Protoxylem elements from mature tuber. ×125.
E.—Secondary xylem vessels from mature tuber. ×125.

PLATE 3

Longitudinal section of stolon tip prior to tuber formation. ×130. (Drawing is a copy of photomicrograph.)



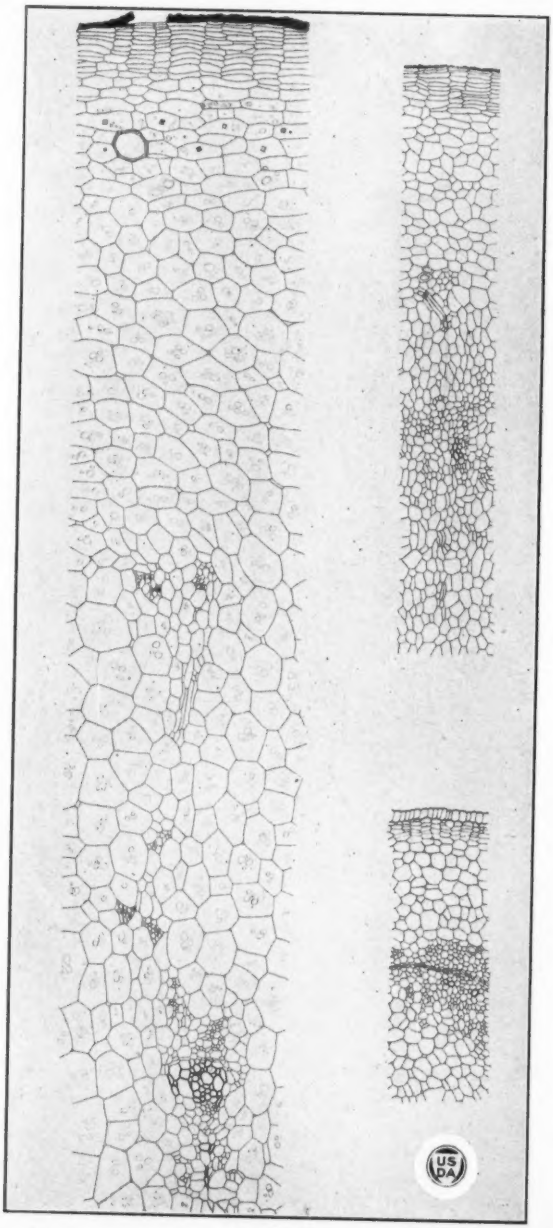


PLATE 4

Stages in the development of the tuber.

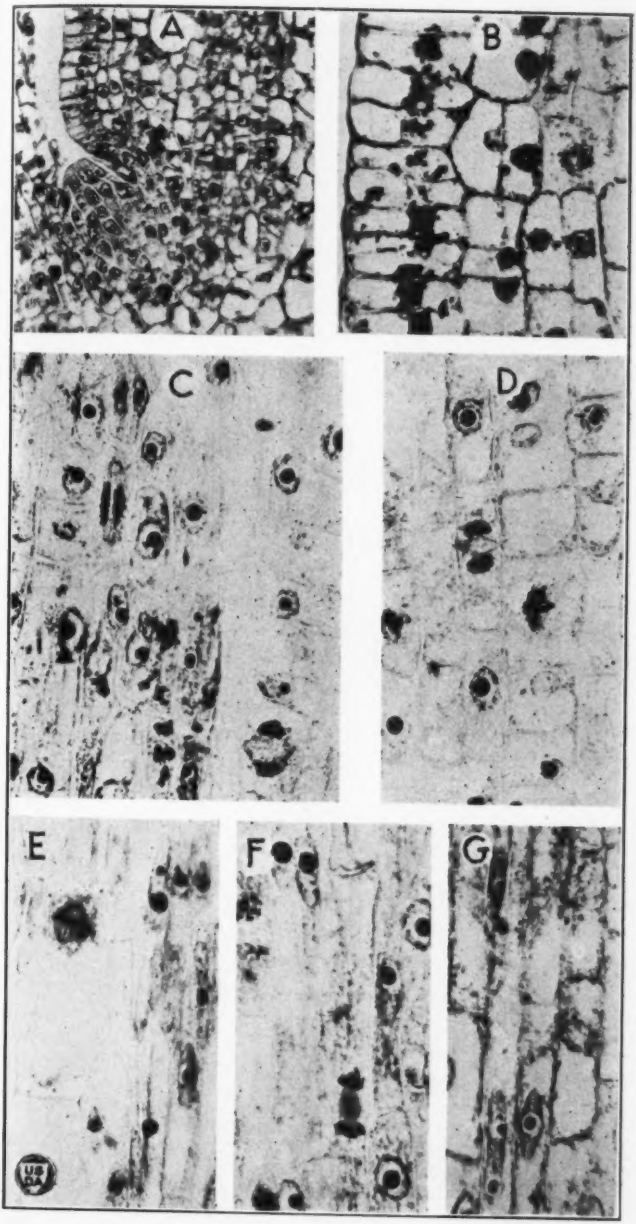
A.—Section through periderm, cortex, pericyclic region and vascular ring of mature tuber. ×45.

B.—The same as in A, except that tuber is only 10 mm. in diameter. ×45.

C.—The same as A and B, but tuber is only 2.5 mm. in diameter.

PLATE 5

A.—Section through tuber bud initial. ×113.
B.—Radial section of epidermis and adjacent cortical cells of stolon tip. ×583.
C.—Mitotic division in young stolon cells. ×583.
D.—Mitotic division—late meta- and telo-phase. ×583.
E.—Mitotic division—metaphase. ×583.
F.—Mitotic division—anaphase. ×583.
G.—Phloem cells with elongated nuclei. ×583.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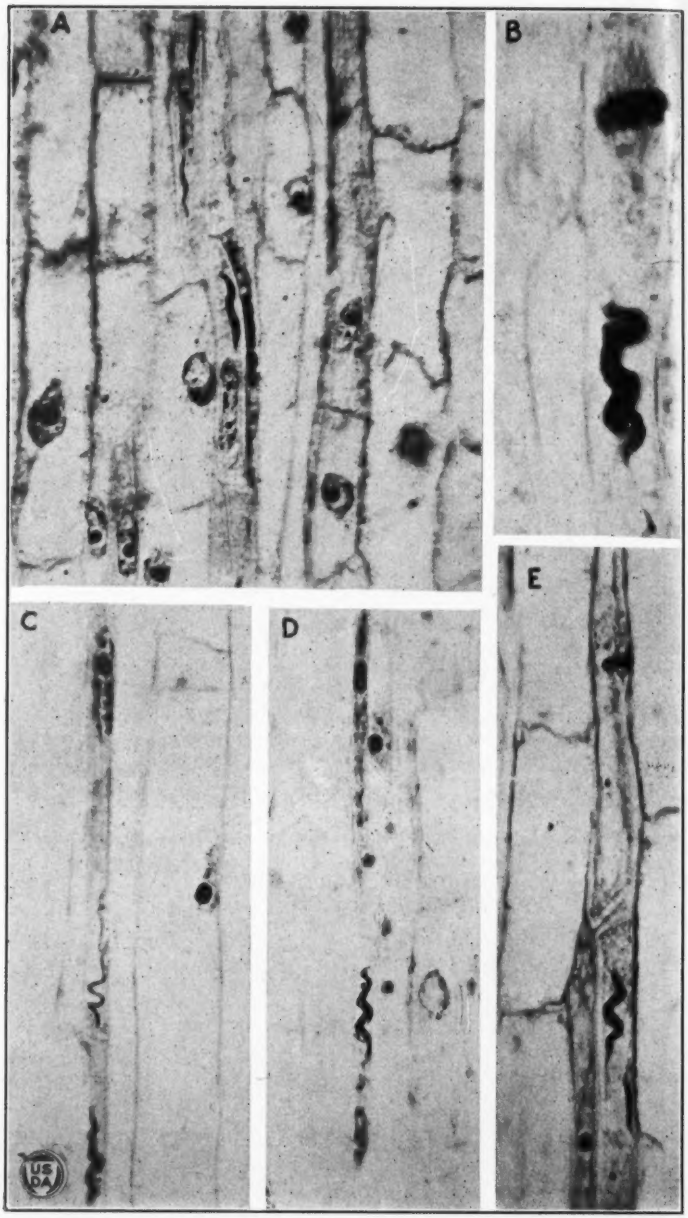
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PLATE 6

A.—Sieve tube with nucleus and slime body. ×800.
B.—Sieve tube with slime body and nucleus in metaphase. ×1500.
C.—Two sieve tubes with large nucleus and two slime bodies. ×800.
D.—Sieve tube with slime body. Notice that the slime body is not homogeneous throughout. ×800.
E.—A more mature sieve tube with a large and small slime body. ×800.

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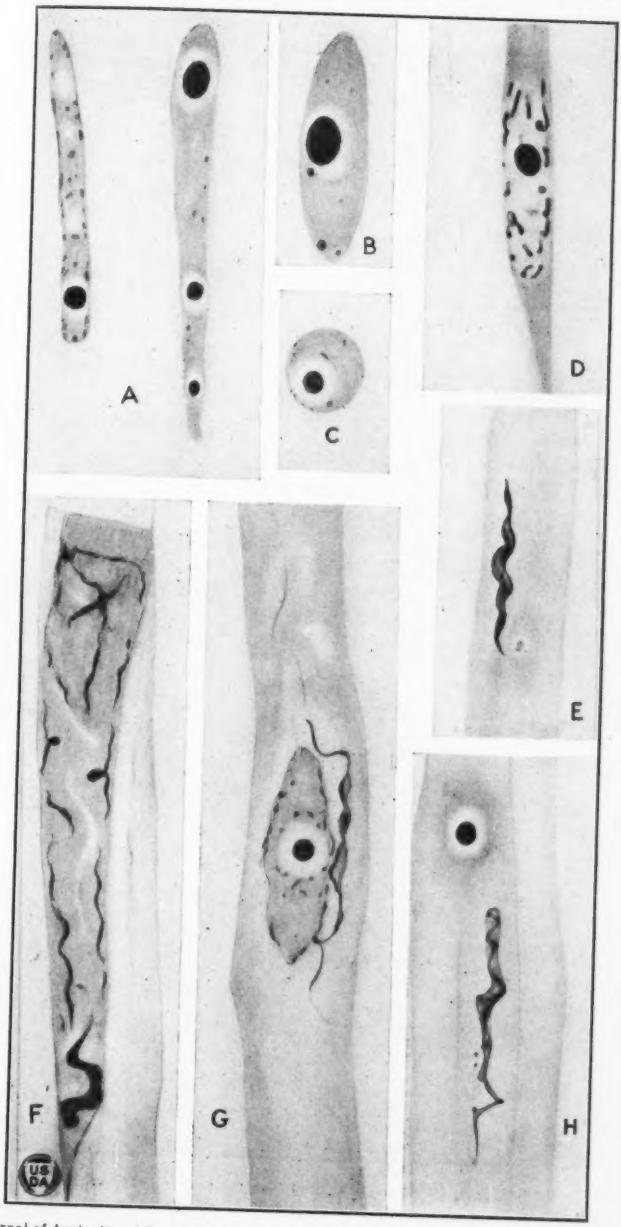
PLATE 7

A.—Two elongated nuclei from cells of procambium.
B.—Typical nucleus of immature vascular cell.
C.—Typical nucleus of the apical meristem.
D.—Elongated nucleus in prophase.
E.—Corkscrewlike body of young sieve tube.
F.—Large aggregate of sieve-tube bodies.
G.—Immature sieve tube with nucleus and sieve-tube body.
H.—Sieve-tube body showing differential staining reaction. Body is in appearance and structure not unlike certain Protozoa.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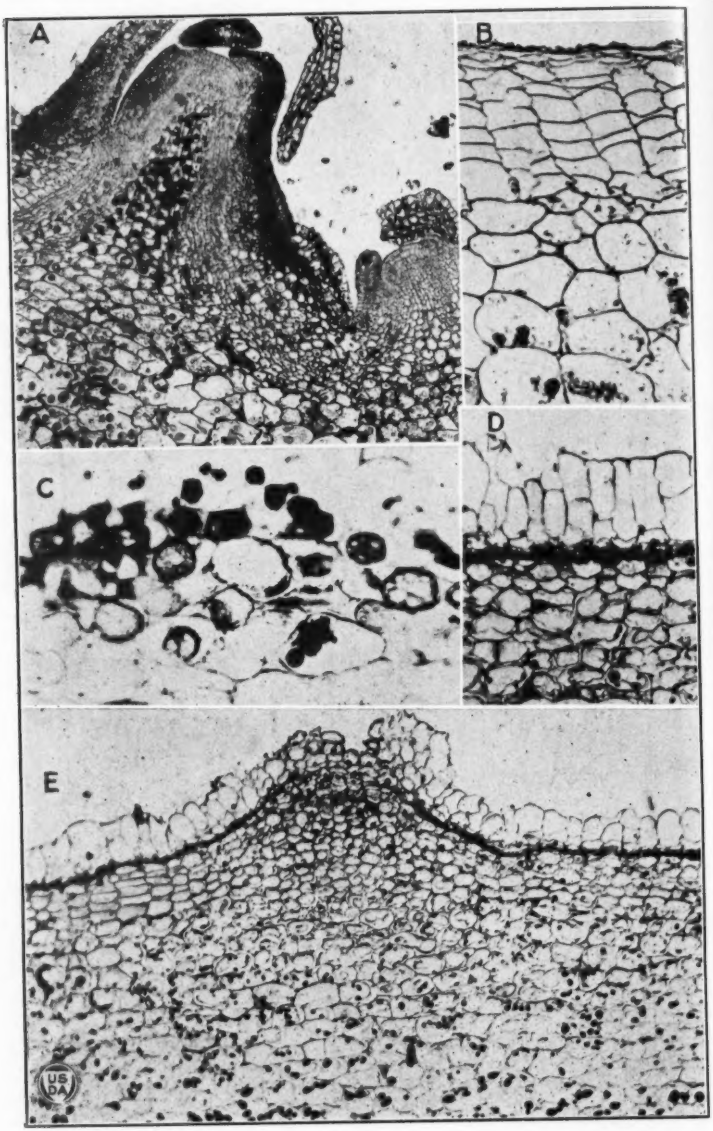
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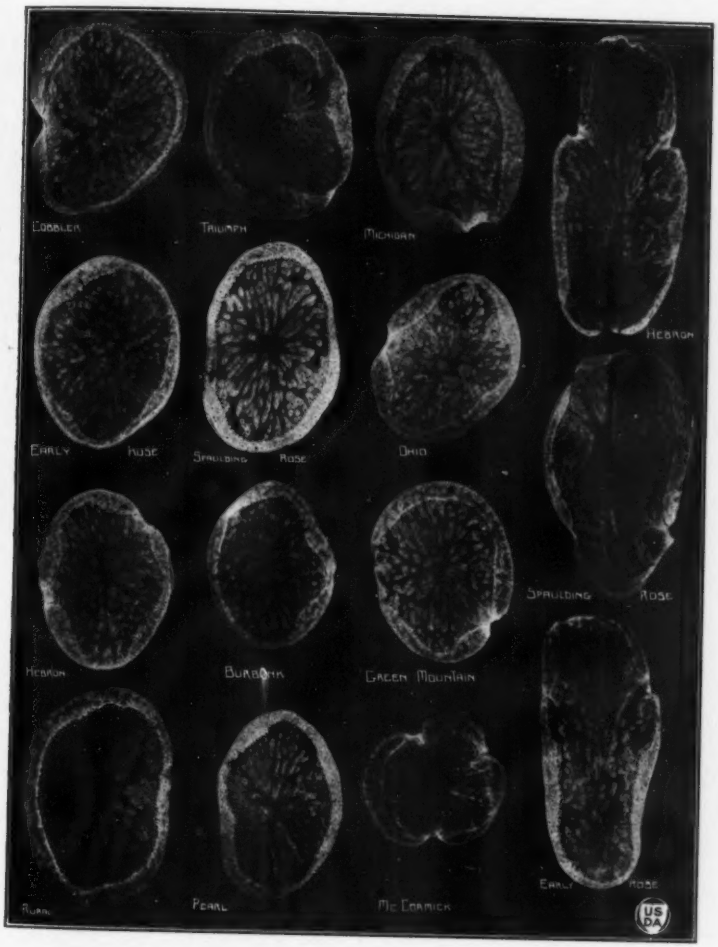
PLATE 8

A.—Tannin vesicles in parenchyma cells of bud. ×74.
B.—Cross section of a normal potato periderm. ×133.
C.—Tannin vesicles in cells of cortex, showing inner structure. ×312.
D.—Cross section of a hypertrophied periderm. ×133.
E.—Cross section of lenticel with hypertrophied periderm. ×133.

PLATE 9

Cross and longitudinal sections of mature tubers. ×¼.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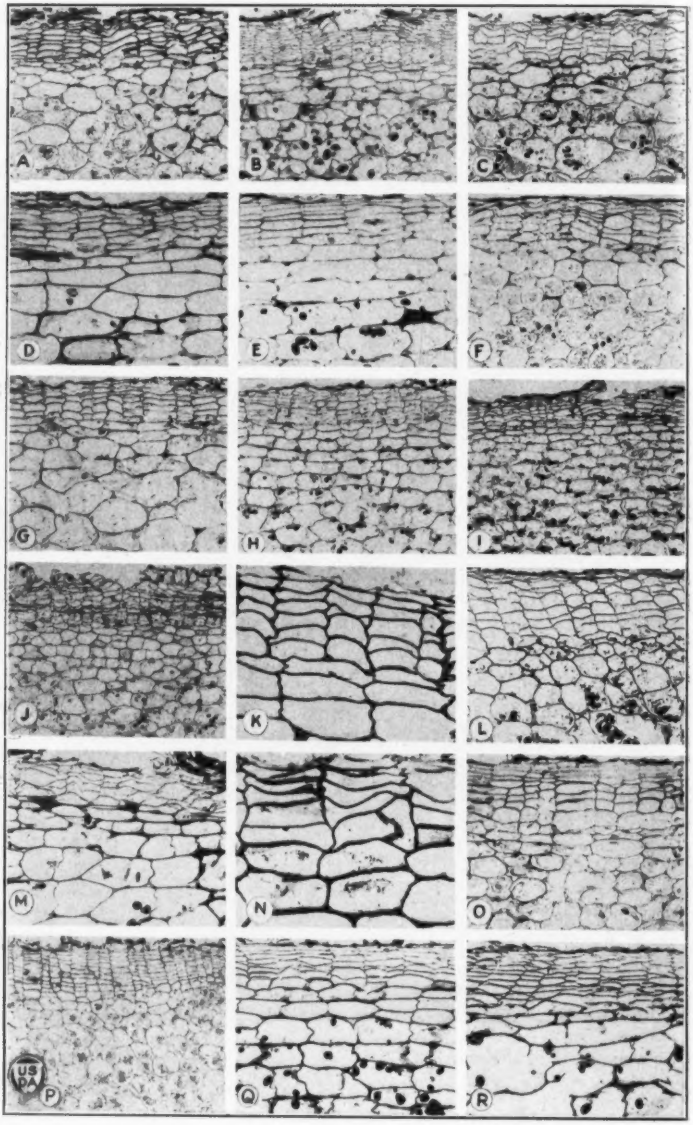
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PLATE 10

Cross sections through the periderm of tubers.

A.—Irish Cobbler..... × 36
B.—Irish Cobbler..... × 36
C.—Bliss Triumph..... × 36
D.—Michigan..... × 36
E.—Rose..... × 36
F.—Rose..... × 36
G.—Ohio..... × 36
H.—Hebron..... × 36
I.—Burbank..... × 36
J.—Burbank..... × 36
K.—Green Mountain..... × 155
L.—Green Mountain..... × 36
M.—Rural..... × 36
N.—Rural..... × 155
O.—Pearl..... × 36
P.—McCormick..... × 36
Q.—McCormick..... × 36
R.—Up-to-date..... × 36

OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON OTHER HOSTS[1]

By NEIL E. STEVENS, *Pathologist, Fruit-Disease Investigations*, and ANNA E. JENKINS, *Assistant Mycologist, Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In the practical control of a plant disease caused by a fungus it is obviously of first importance to know whether the causal organism is confined to a particular host or grows, either as a parasite or saprophyte, on other hosts in the same locality. In the present paper evidence is presented to show that the cane blight fungus, an active parasite on the cultivated currant, occurs also on at least two unrelated hosts.

CURRANT CANE BLIGHT

The disease of cultivated currants, now generally known as cane blight, was first reported more than 30 years ago by Fairchild [2] who found it in the Hudson Valley about Highland and Milton, N. Y. Of subsequent investigations, the most important is that made by Grossenbacher and Duggar [3] in the same region during the period from 1907 to 1911. One of the writers has had this disease under observation since 1917 and has made culture and inoculation experiments in various parts of the eastern United States.

The most conspicuous symptom of this disease and the one from which its common name is derived is the wilting of the leaves and fruit of infected plants. This apparently sudden wilting occurs most frequently during the period when the fruit is being matured and is usually caused by the girdling of the main stem by the fungus which has grown in from a lateral branch.

Infection generally occurs through a terminal or lateral bud and the parasite develops basipetally, invading all woody structures. Young shoots which have become infected usually die at the distal ends during the same season. The growth of the fungus apparently continues throughout the growing season during which large branches or even main stems may be blighted at any time. However, as stated above, the wilting is usually most conspicuous just before the fruit ripens, perhaps because of the greater demands on the moisture supply at that time. The disease may be recognized with a fair degree of certainty by the peculiar blackened appearance it gives to the wood and pith. So far as has been determined, the roots are not infected.

The disease is known to occur in Massachusetts, Connecticut, New York, Ohio, Pennsylvania, New Jersey, Maryland, and Virginia. Currants are grown only locally in Maryland and Virginia. The disease is very serious in New Jersey and has been an important factor in the reduction of the currant acreage in that State during recent years. It is

[1] Received for publication Jan. 27, 1924.

[2] FAIRCHILD, D. G. NOTES ON A NEW AND DESTRUCTIVE DISHASE OF CURRANT CANES. (Abstract) Bot. Gaz. 16: 262. 1891.

[3] GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHAERIA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18: 115-190, illus. 1911.

destructive also, especially during certain seasons, in the Hudson Valley of New York and in southern Connecticut. Farther north, however, it seems to be of much less importance, and has not yet been found to be serious in northern New England or in Canada. The fungus has not been found in Alaska, though currants are commonly grown there.

No effective method of control for currant cane blight has been developed. Systematic cutting out and burning of the diseased parts has, however, served to keep the disease down to a point where currants are commercially profitable even though the disease is commonly present.

THE CAUSAL ORGANISM

Grossenbacher and Duggar showed by inoculation experiments that currant cane blight was caused by a fungus to which they gave the name *Botryosphaeria ribis*. They also called attention to the frequent occurrence on currant canes of a morphologically similar fungus which is not parasitic and which they designated as *Botryosphaeria ribis* forma *achromogena*. This form name was chosen because the saprophyte lacks the most conspicuous cultural character of the parasite, namely, the production on starch paste or other starchy media of a bright pink color during the first three or four days of growth.

The observations of the senior writer as to the causal organism have fully confirmed those just reviewed. Currant cane blight seems to be everywhere associated with the chromogenic Botryosphaeria, and infection with the chromogen is readily secured from inoculations made at the right time.[4] On the other hand, the results of inoculations with the nonchromogenic form have been uniformly negative. Grossenbacher and Duggar consider the two forms morphologically practically identical, the ascospores measuring 16–23μ by 5–7μ and the pycnospores 16–31μ by 4.5–8μ. The material examined by the writers varies through somewhat wider limits than those given by Grossenbacher and Duggar, perhaps due to the fact that it has been derived from a wider geographical range. Comparison of our material, however, both with the authentic material distributed in Bartholomew's *Fungi Columbiani*, Numbers 3408 and 3409, and with the original slides of Grossenbacher and Duggar now preserved at the Geneva Experiment Station, as well as culture and inoculation work, leaves no doubt that the fungus described by them is identical with that used in our inoculation work on currant.

OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON HORSE-CHESTNUT

Our work on the currant cane blight fungus forms a part of the extensive studies of the fungi belonging to this group which have been carried on for more than 15 years by Shear and his associates.[5] During this period, as stated by Shear and Beckwith,[6] fungi which closely resembled *Botryosphaeria ribis* morphologically have been secured from several hosts. However, although one strain collected on Viburnum showed chromogenesis when cultured on starchy media, no direct evidence of the occurrence of the currant cane blight fungus on hosts other than Ribes sp. was secured until 1921.

[4] Grossenbacher, J. G., and Duggar, B. M. op. cit., p. 137.

[5] Shear, C. L. Life history of Melanops quercuum (Schw.) Rehm forma vitis Sacc. (Abstract.) Science 31: 748. 1910.

[6] Shear, C. L., and Beckwith, A. M. Life histories of Melanops. (Abstract.) Phytopathology 6: 109. 1916.

In the fall of that year one of the writers, during a collecting trip through the currant-growing regions of New York, picked from a horse-chestnut tree (*Aesculus hippocastanum*) growing on a lawn between Milton and Marlborough, a dozen or more mature, apparently healthy fruits. Not more than 20 feet from the tree was a good sized currant patch in which cane blight was abundant. The fruits were collected, however, with the expectation that a nonchromogenic fungus of this type would develop, as one had been repeatedly secured on several successive years from Aesculus fruits collected in the District of Columbia. The fruits from New York were taken to Washington and placed in a moist chamber for several weeks, when they became covered with the pycnidia typical of *Botryosphaeria ribis*. Single spore cultures made from this material showed, however, the chromogenesis hitherto associated only with the currant parasite. Repeated comparative cultures were made and the horse-chestnut fungus proved to be indistinguishable from the known parasite from currant.

Late in December of that year 24 healthy 3-year-old currants of the Wilder and Red Dutch varieties were secured from northern New York and planted in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. When the plants had reached the stage most favorable for infection,[7] one-third of them, about 30 in all, were inoculated with the horse-chestnut fungus, an equal number with the parasite from currant, and the remainder were maintained as controls, that is, were wounded with a sterile knife but not inoculated.

The results were conclusive. Nineteen of the 30 inoculations with the fungus from horse-chestnut developed the typical cane blight and were dead by the middle of May. Only 16 of the twigs inoculated with the fungus from currant developed the disease, but this difference is no doubt accidental, as the percentage of infection is as high as that usually secured in our previous work out of doors. None of the control plants became diseased. Single spore cultures from the pycnospores which matured later on the dead limbs showed that the chromogenic fungus was present in every case. All the plants which had been inoculated with the horse-chestnut fungus were transplanted during the latter part of June to a shaded place isolated from any other currants.

OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON ROSE

In October, 1922, diseased canes from a plant of *Rosa setipoda* growing in the rose garden at the Bell Station, Maryland, were referred by the Federal Horticultural Board to one of the writers for identification of the fungus present. Microscopical examination showed that pycnidia of the Dothiorella type were fruiting on the stems, which were severely cankered. In cultures on corn meal chromogenesis similar to that associated with the cane blight fungus was evident after about 24 hours. Single pycnospore cultures were likewise chromogenic. This was the only observation of the disease in 1922, but late in March, 1923, a few discolored areas were found on the canes of three large plants, one of which was the *Rosa setipoda* plant from which the first collection was made. Pycnidia, bearing pycnospores similar to those present on the October material, were distributed over the lesions and from these the chromogen was again isolated.

[7] GROSSENBACHER, J. G., and DUGGAR, B. M. *op. cit.*, p. 137.

The cultures from the fungus on rose were indistinguishable from those of the cane blight fungus and the pycnospores were also in agreement with those of *Botryosphaeria ribis*. Therefore it seemed very likely that we were again dealing with the currant blight fungus on another unrelated host. Moreover, on the rose the fungus was apparently parasitic. Accordingly, it was planned to conduct inoculation experiments with roses as well as currants.

The inoculation experiments on currant were conducted as described above for the material from horse-chestnut, except that 2-year-old currant bushes were used, and no parallel inoculations were made with the fungus from currant. Cultures made from a single pycnospore were used. More than half the twigs inoculated with the rose fungus developed typical cane blight and reisolations showed the presence of the chromogenic fungus in every case. The controls remained healthy.

PARASITISM OF CURRANT CANE BLIGHT FUNGUS ON ROSE

For the inoculations on the rose, 12 potted plants of the variety Columbia (Hybrid Tea) which had been propagated from cuttings were used. On April 2, 1923, 4 of the plants were inoculated with subcultures from those used in the currant inoculations, 4 with cultures made from the specimens collected in March, while the remaining 4 plants were kept for controls. The method of making the inoculations was the same as for currant. About two weeks after the inoculations were made all of the inoculated plants showed infection and the imperfect stage of the Botryosphaeria was fruiting on some of the stems. A week later the foliage above the point of inoculation had become yellowish green and was beginning to wilt, and by the last of the month it was dead and dry (Pl. 2). The controls remained healthy and the wounds which had been made in the stems by the sterile scalpel had healed. The perfect stage of the Botryosphaeria was observed on a number of the infected plants two months after the plants were inoculated. Cultures made from the ascospores, as well as the earlier reisolations from infected stems, were chromogenic and produced the imperfect stage of the fungus in about three weeks. Thus the pathogenicity of the fungus on the rose was established.

When the rose garden was again visited late in June a number of plants, including the three on which the cankers were present in March, were found to be severely infected. Many canes 6 or 7 feet in height were killed to the ground, the brown, withered foliage being still attached. The pycnidial pustules of the Botryosphaeria were fruiting abundantly on these blighted stems and on one of the specimens brought to the laboratory immature perithecia were present.

On October 25 another survey of the garden was made and the disease was observed on still other varieties. The perfect stage of the fungus was fruiting abundantly on many of the specimens taken at this time and single ascospore isolations developed the typical chromogen. On this date the Botryosphaeria was collected for the first time on an American species, *Rosa pratincola*. Otherwise the disease has been observed only on rose species from China or their hybrids, as follows: *R. adenosephala*, *R. banksiopsis* ×, *R. bella* ×, *R. bella* × *moyesii*, *R. caudata*, *R. heritierana*, *R. moyesii*, *R. moyesii* × *canina*, *R. multabracteata* ×, *R. setipoda*, and a number of other varieties. There is nothing to indicate, however, that the fungus itself was imported from China. Cane blight occurs commonly on

currants in the near neighborhood, from which the fungus might easily have spread to the roses.

The symptoms produced by the currant cane blight pathogene on the rose differ somewhat from those on the currant. In some cases the entire cane is not blighted and the lesions remain as definite cankers on the stems (Pl. 1 A, B, E). The small pimple-like fruiting bodies of the fungus are distributed over the surface of the cankered bark which is of a characteristic "seal brown" [8] color. The region surrounding the lesions is often "vinaceous brown" shading to "dark Corinthian purple" next to the edge of the canker. Recently blighted canes, upon the development of the fruiting structures of the fungus, become speckled with slightly raised golden brown dots (Pl. 1, C). As the fungus develops, considerable areas may present this golden brown color which, with the natural sheen of the bark, makes the cane appear as though gilded. These surfaces later become dull, owing to the further development of the dark stromata beneath. In some cases the two shades of brown are arranged more or less concentrically as shown in Plate 1, D. The diseased canes may become so swollen that the bark is roughened and split longitudinally (Pl. 1, E).

The manner in which the pathogene gains entrance to the rose is not known but it seems very likely that infection usually occurs through a bud or young shoot from which it passes to the main stem (Pl. 1, A and B). Small lesions have been observed, however, where no buds, shoots, or visible abrasions were present. As in the currant, the fungus evidently penetrates all parts of the stem, having been isolated from both the cortex and wood in which the mycelium occurs intercellularly.

IDENTITY OF THE FUNGI FROM THE THREE HOSTS

Until the autumn of 1923, comparisons of the chromogenic fungi from horse-chestnut, rose, and currant were based on cultural characters, pycnospore size and their parasitism on currant. During July of that year, however, ascospores developed on the inoculated rose plants, and in September and October mature ascospores were found on the currants which had been inoculated with the fungus from rose and from horse-chestnut, respectively. A careful comparison of the material from the various hosts was now possible.

In Grossenbacher and Duggar's description of the currant cane blight fungus, the stromata are described as being 1–4 mm. in diameter, usually 2–3 mm. The perithecia measured 175-250μ in width. The asci were 80–120μ by 17–20μ and the ascospores 16–23μ by 5–7μ. They describe the pycnidia as having about the same size as the perithecia, and the pycnospores as measuring 16–31μ by 4.5–8μ.

The perithecia of the fungus which developed on currant and rose from inoculations made with material from the various hosts were of practically the same size and agreed closely with those of the parasite which had developed from natural infections, although because of the relation of the perithecial wall to the surrounding tissues of the fungus, it is difficult to make accurate comparative measurements.

Measurements of over 200 ascospores of the currant cane blight fungus from various localities gave a range in size from 14–27μ by 5–10μ, though

[8] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

the great majority of the spores were within 16–23μ by 6–8μ. From 25 to 50 ascospores were measured from each of the following sources: Rose from natural infection, rose from inoculations with fungus from rose, currant from inoculations with fungus from rose, and currant from inoculations with fungus from horse-chestnut. In all cases the spores agreed closely in size with those from currant. The extreme range was from 15–25μ by 5–10μ, but the spores fell mostly within the limits 17–22μ

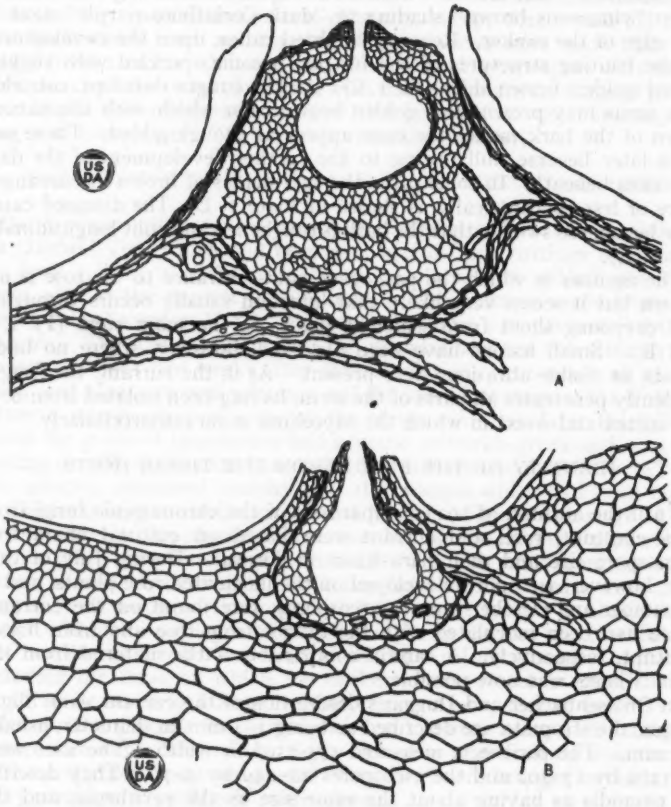


FIG. 1.—Semidiagrammatic section through single mature perithecium of currant cane blight fungus A, on currant, B, on rose. Note greater amount of stromatic tissue about perithecium which developed on currant. The host tissue is indicated by heavier lines. Both perithecia resulted from inoculations made with subcultures from the same pycnospore. X 140.

by 6–8μ. The pycnospore measurements were equally satisfactory. In about 200 spores measured, the range was 14–31.5μ by 4–7.5μ, mostly 18–23μ by 5–7μ, the pycnospores from the various hosts being in close agreement.

The size of the stromata was much more variable and is apparently directly influenced by the thickness and character of the bark within which they grow. The writers have observed on a single currant bush perithecial stromata of this fungus which varied in size from about 1 by

2–3 mm. on the smaller branches to 3–3.5 by 5–8 mm. near the base of the older canes where the bark was 2.5 mm. in thickness. Moreover, the host apparently affects to some extent the amount of stromatic tissue which develops about the perithecium. In the present study it was evident that the perithecia which matured on the inoculated rose plants had associated with them much less stromatic tissue than those which matured on inoculated currant plants (fig. 1, A, B). That the inoculum was identical in the two cases is assured by the fact that subcultures from a single ascospore culture were used in both cases. This observation alone would suggest that mere size of stroma is an unreliable character for the identification of species in this group.

SUMMARY

Currant cane blight is now known to occur in Massachusetts, Connecticut, New York, Ohio, Pennsylvania, New Jersey, Maryland, and Virginia, but is less severe in the northern portions of this region.

The disease is caused by a fungus to which the name *Botryosphaeria ribis* was given by Grossenbacher and Duggar, who first demonstrated its pathogenicity.

A fungus identical morphologically and in cultural characters with the currant cane blight fungus has been collected by the writers on horse-chestnut and rose.

Inoculations on currants with the fungus from both these hosts have produced typical cane blight.

The fungus is apparently parasitic on several varieties of cultivated roses, and its pathogenicity has been established by inoculations on at least one variety.

The size of the stromata in this fungus is apparently directly affected by the thickness of the bark in which it develops as the stromata are smaller in thin currant bark than in thick bark, and uniformly smaller on rose than on currant.

In view of the agreement of the fungi from the different hosts in cultural characters, morphology, and parasitism, the conclusion seems amply warranted that they are identical, and that the fungus hitherto known only as the causal organism of currant cane blight does actually occur on other hosts.

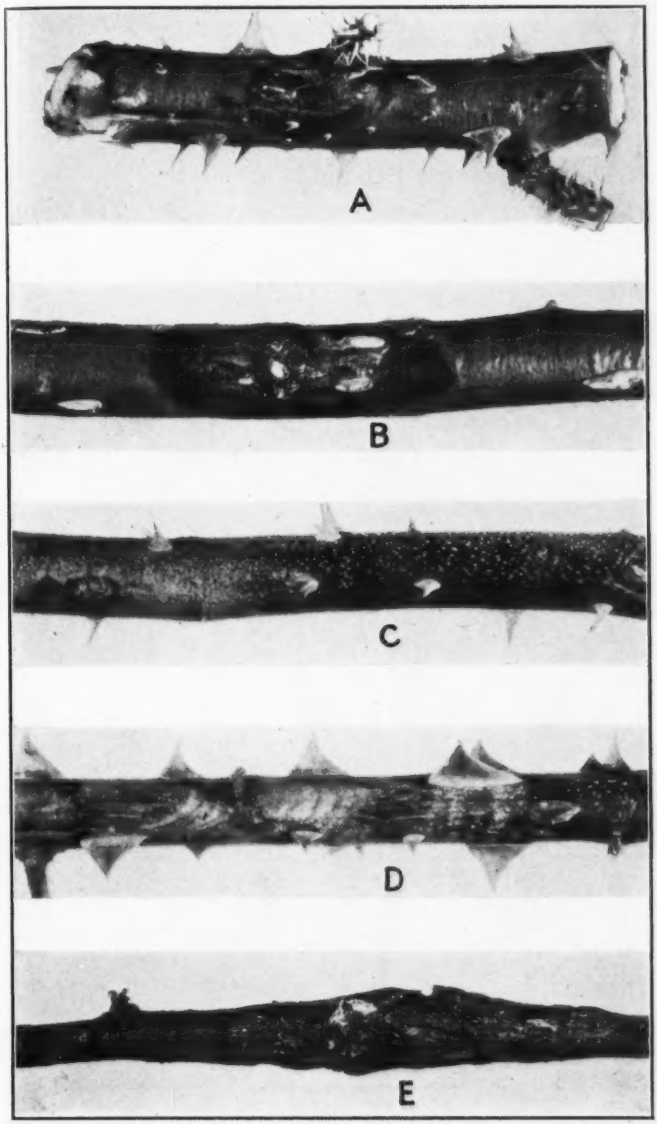
PLATE 1

Portions of rose canes affected with *Botryosphaeria ribis* G. & D.

A, B.—Cankers as observed on March 21, 1923. The causal parasite has apparently entered through the small shoots at the center of the lesions.

C, D.—Blighted canes on June 28, 1923. The pycnidial pustules of the fungus appear as lighter colored areas distributed over the surface of the bark.

E.—Cankered portion of a cane of *Rosa setipoda* showing enlarged stem with the bark very much roughened and broken. Specimen received from the Federal Horticultural Board, October, 1922.


A
B
C
D
E

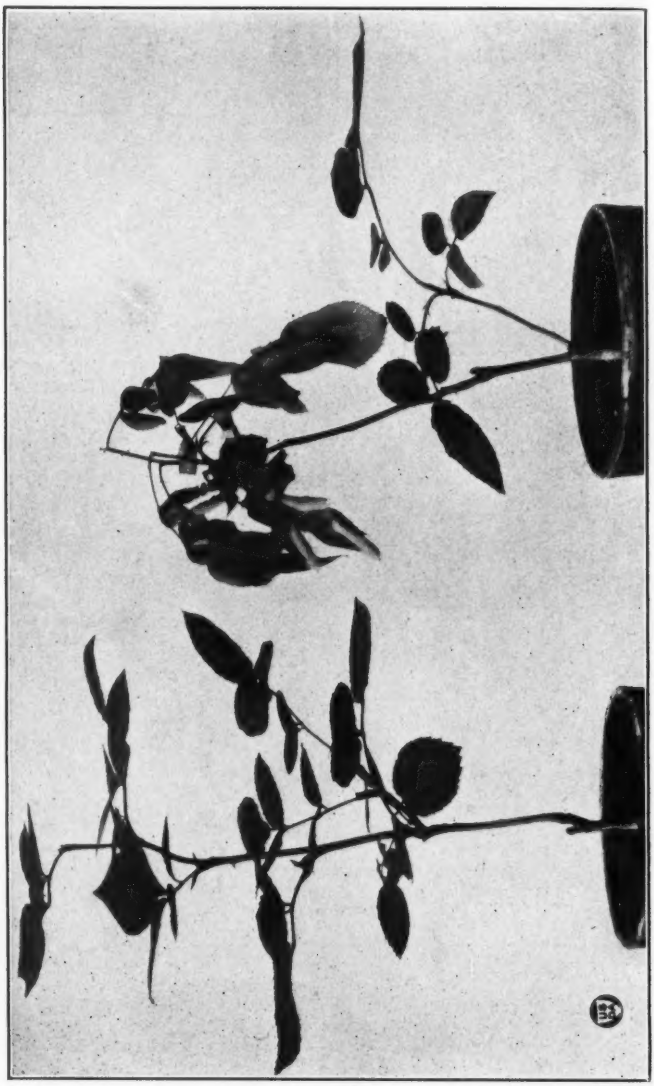


PLATE 2

On the right, rose plant of the variety Columbia four weeks after inoculation with *Botryosphaeria ribis* G. & D. The midrib of a leaf from the healthy branch crosses the main stem near the point of inoculation. Control on the left.

ELEMENTAL COMPOSITION OF THE CORN PLANT[1]

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INTRODUCTION

Our knowledge concerning the elemental composition of many of the more common agricultural plants is rather limited and fragmentary. The data on this subject have been obtained frequently from material that has been collected under no stated conditions and the earlier determinations are now open to criticism on account of the questionable accuracy of the methods of analysis employed. An accurate elemental analysis of a few mature plants of a given crop grown under well-defined conditions of soil and climate should furnish data from which the amount of the various elements removed from the soil by the crop could be fairly accurately estimated. For this reason it was thought advisable, in some of the experimental work with corn at the Kansas Agricultural Experiment Station, to make an elemental analysis of the plant. With this in view material was collected from mature Pride of Saline corn plants grown in the field at Manhattan, Kans., during the summer of 1920.

CULTURAL METHODS

The plants used in this experiment were grown on plots that had been continuously cropped to corn for about 10 years. The soil was a fertile sandy loam and showed little difference in texture in the first 4 feet. The ground was plowed to a depth of 6 inches in the late fall and received no other cultivation until the following spring, when it was worked into good condition just previous to planting. The seed was surface planted on May 12 in rows 42 inches apart, and after the plants had reached a height of about 3 inches, they were thinned in the row to a distance of 2 feet between each plant. The plot was kept free from weeds by hoeing, and with the exception of a shallow cultivation on June 3 the soil was not stirred during the growing season. Since no visible signs of wilting were evident at any time during the summer, it is assumed that the supply of water in the soil was adequate. A summary of the general climatic conditions prevailing during the growing season is given in Table I.

[1] Received for publication Mar. 1, 1924. Published with the approval of the Director as paper No. 219, Department of Botany, and paper No. 106, Department of Chemistry, Kansas Agricultural Experiment Station.

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TABLE I.—*Summary of the climatic conditions at Manhattan, Kans., for the growing season of 1920*

Month.	Days (inclusive).	Air temperature (° F.).					Precipitation.	Evaporation from free water surface.
		Average of—			Maximum.	Minimum.		
		Mean.	Maximum.	Minimum.				
							Inches.	*Inches.*
May	1–5	61	71	51	74	42	.....	0.568
Do	6–10	61	73	50	83	46	0.64	.783
Do	11–15	59	68	51	81	40	.25	.713
Do	16–20	61	72	51	85	45	.04	.591
Do	21–25	70	79	61	88	47	.48	.801
Do	25–31	69	79	58	92	54	.17	.810
June	1–5	65	77	54	82	40	.34	1.077
Do	6–10	78	90	67	98	58	.....	1.405
Do	11–15	84	96	73	99	72	.....	1.983
Do	16–20	68	78	59	91	55	.04	1.253
Do	21–25	70	87	55	90	47	.76	1.418
Do	26–30	84	79	61	98	69	.82	1.776
July	1–5	83	96	70	100	61	.02	1.339
Do	6–10	71	85	57	89	54	.15	1.422
Do	11–15	77	90	65	95	55	1.02	1.520
Do	16–20	79	93	65	97	61	.....	1.290
Do	21–25	82	97	67	105	63	.....	1.725
Do	26–31	72	85	59	94	51	3.64	1.143
August	1–5	74	86	63	90	58	.....	1.128
Do	6–10	79	94	61	96	57	.....	1.080
Do	11–15	70	84	56	90	47	.12	1.232
Do	16–20	71	87	53	91	48	5.18	.857
Do	21–25	68	79	58	85	51	.....	1.172
Do	26–31	73	84	62	92	56	.87	1.306

COLLECTION AND PREPARATION OF MATERIAL

STEMS, LEAVES, AND GRAIN

Five plants were selected and harvested on September 2, when the grains were in the late-dough stage and were well glazed and dented (Pl. 1, A). At this stage of development it was thought that the plants had removed from the soil most of the minerals of which they were capable, although there would probably be some slight rearrangement of materials in the various plant parts before full maturity. Furthermore, at this stage the leaves were all attached to the plants (Pl. 1, B) and green, so that little or no material had been lost from them by leaching. It is worthy of note that a gentle rain fell during the night of September 1, so that the leaves and stems were especially free from any adhering dirt when they were harvested. The plants were cut off at the surface of the ground and their green weight at once determined. A general description of each of the five plants at the time of harvesting is given in Table II.

As soon as the green weight of each plant was determined, the stem, leaves, and grain were separated, ground or cut into convenient pieces, and placed in a hot air drying oven at 105° C. for 24 hours. The material was then transferred to glass jars and sealed until it was ready to be

pulverized for chemical analysis. In the preparation of material the husks were placed with the leaves and the tassel with the stem. The cobs of the five plants were ground together and the analysis made of this mixture, so that the variation in the composition of these organs was not determined.

TABLE II.—*General description of the five corn plants at the time of harvesting*

Plant No.	Total green weight.	Dry weight of—					Percentage of moisture in plant.	Height of plant.	Length of ear.	Number of leaves.
		Entire plant, excluding the roots.	Stems and tassel.	Leaves[a] and husks.	Grain.	Cob.				
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*		*Inches.*	*Inches.*	
1	2, 540	776. 3	154. 3	209. 4	320. 0	92. 6	69. 4	102	12	15
2	2, 923	791. 1	191. 2	252. 4	272. 2	75. 3	72. 9	96	13	16
3	2, 277	659. 5	161. 0	195. 8	232. 0	70. 7	71. 0	101	12	14
4	2, 716	745. 8	233. 0	235. 5	212. 0	65. 3	72. 5	108	11	14
5	3, 095	899. 3	263. 5	279. 7	268. 8	87. 3	70. 9	120	13	17
Average	2, 710	774. 4	200. 6	234. 6	261. 0	78. 2	71. 4	105	12	15

[a] Including sheaths.

ROOTS

It is practically impossible to collect all of the roots of a corn plant grown under crop conditions in the field since one is unable to distinguish or separate the finer roots that are interwoven with those of the adjacent plants. In order to obtain the entire root system, plants of the same variety of corn as that grown in the field were grown singly in soil in large galvanized iron cans. These cans contained a sufficient volume of soil to grow plants to maturity that in appearance were the equal in every regard of those growing under the conditions of the surrounding field. The methods used in growing plants in such containers have been previously described by Miller (*8*, *9*)[1] and will not be discussed here. When the plants in the cans had reached maturity, the aerial parts were harvested and discarded and the roots collected in the following manner: The soil contained in the can was emptied upon a cleared space and the larger roots removed by careful sorting. The soil with the smaller roots was then placed in vessels and covered with a large excess of water which was stirred vigorously until the soil had disintegrated into fine particles. As soon as the stirring ceased, the soil settled to the bottom of the vessels while the remnants of roots floated to the surface and were removed by skimming with a fine sieve. The process was repeated several times until all the roots had been separated from the soil. The roots thus obtained were washed carefully until, in so far as could be seen, they were free from all soil particles, dried in a hot-air oven at 105° C. for 24 hours, and stored in sealed jars until chemical analyses could be made. A representative root system of one of the five obtained after this manner is shown in Plate 1, C.

[1] Reference is made by number (italic) to "Literature cited," p. 859.

CHEMICAL METHODS

The chemical determinations of the various elements with the exception of sulphur and phosphorus were made, in general, according to the methods for the analysis of plant material recommended by the Association of Official Agricultural Chemists. The sulphur and phosphorus were determined after the following method described by Latshaw (7). One to two grams of the sample, or a sufficient amount to give a precipitate of not less than 30 mgm. of barium sulphate, were weighed into a 250 cc. low form Pyrex beaker. To this were added 7.5 cc. of a magnesium nitrate solution,[3] care being taken that all the material was brought in contact with the solution and heated on an electric hot plate (180° C.) until no further action took place. The beaker was transferred while hot to an electric muffle and allowed to remain at low heat (muffle not showing any red) until the charge was thoroughly oxidized and no black particles remained. If necessary, the charge was broken up and again returned to the muffle. The beaker was removed from the muffle and allowed to cool. Water was added, then hydrochloric acid in excess, the solution brought to a boil, filtered and washed thoroughly. This solution was then diluted to 200 cc., heated to boiling, and a 10 per cent barium chlorid solution added in small quantities until no further precipitate was formed. The boiling was continued for five minutes, after which the liquid was allowed to stand for five hours or longer in a warm place. The liquid was then decanted on an ashless filter or a tared Gooch crucible previously heated, the precipitate treated with 15 to 20 cc. of boiling water, transferred to the filter and washed free of chlorids with boiling water. The precipitate was ignited and weighed as barium sulphate. The filtrate obtained in the sulphur determination was evaporated to 75 cc. and the phosphorus determined by the method recommended by the Association of Official Agricultural Chemists.

The figures in the various tables representing the amounts of oxygen include the oxygen obtained by the usual procedure in organic analysis plus the oxygen that was a part of the various mineral elements of the ash when they were converted to their oxids. The figures thus represent the oxygen of both organic and inorganic combination.

The determinations were all made in duplicate or triplicate and whenever any striking differences appeared in the analysis of the individual plants a careful redetermination was made to verify the results. Any marked differences recorded in the composition of the same organ of the different plants are due to individual variations and not to discrepancies in the chemical methods used.

DISCUSSION OF EXPERIMENTAL DATA

In order to determine the variation in the composition of the different plants and their organs a separate analysis was made of the leaves, stem, and grain of each of the five plants. A separate analysis was also made of each of the five root systems. Determinations were made for carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, silicon, aluminum, chlorin, and manganese. The amount of each of the elements that compose the leaves, stem, and grain of each of the five plants is expressed in percentage of dry weight and in the actual amount, in grams, in Table III.

[3] Made by dissolving 320 gm. of calcined magnesia in nitric acid, avoiding an excess of the latter. Calcined magnesia is then added slightly in excess, the solution boiled, filtered from the excess of magnesia, iron aluminia, etc., and diluted to two liters.

In Table IV the composition of the five root systems is expressed in the same manner. The data in Table III shows a striking uniformity in the elemental composition, expressed in percentage of dry weight, of the leaves, stem and grain of each of the five plants analyzed. The only marked exceptions in this regard were the rather wide variations in the percentages of oxygen and chlorin. This was true, however, only of the stems and leaves since the percentages of these two elements in the grain were very uniform. The percentages of several of the mineral elements in the five root systems showed considerable variation which may have been due, in part, to the unequal distribution of minute particles of soil adhering to or embedded in the exterior tissue of the roots.

Although the percentage elemental composition is uniform in a like organ of the different plants, the actual amount of a given element expressed in grams varies considerably, as shown in the latter part of Table III. This is due to the fact that plants which are grown under the same conditions in the field and which seem to be uniform in size and general appearance show marked variations in dry weight and in the distribution of this matter in the various organs of the plants.

DISTRIBUTION OF THE ELEMENTS IN THE ORGANS OF THE PLANT

The total dry weight of the leaves, stem, grain and cobs of each of the five corn plants analyzed was 776.3, 791.1, 659.5, 745.8 and 899.3 gm., respectively. The average amount of dry matter in the aerial parts of the plants was thus 774.4 gm., of which 30.2 per cent was in the leaves, 26 per cent in the stem, 33.7 per cent in the grain and 10.1 per cent in the cob. This proportion of dry matter in the various organs corresponds closely to the observations of Smith (*11*) on corn grown in Michigan. He found that 22 per cent of the dry matter of the plant above ground was in the leaves, 32 per cent in the stalks and 46 per cent in the ears. Table IV shows that the dry weight of each of the five root systems isolated was, respectively, 59.5, 62.4, 60.7, 53.9, and 66.0 gm., with an average weight of 60.5 gm. The weight of the dry matter of the roots was thus 7.81 per cent of the dry weight of the leaves, stem and ear. The weight of the roots obtained in this experiment is in accord with unpublished data that have been obtained in numerous experiments with the corn plant at the Kansas Agricultural Experiment Station. These data show that the dry weight of the roots of a mature Freed White Dent, Kansas Sunflower, Reid Yellow Dent or Pride of Saline corn plant was between 7 and 8 per cent of the dry weight of the portion of the plant above ground.

The values for the weight of the dry matter of the roots of corn are much higher than those given by Hornberger (*3*) and Schweitzer (*10*). Their results show that the dry weight of the roots of mature corn plants grown in the field did not exceed 3 per cent of the dry weight of the plants. They state, however, that the methods used by them to isolate the root systems were unsatisfactory since there was no degree of certainty that they had obtained all the roots.

The average percentage composition of the different organs of the five plants is shown in the first half of Table V. The average weight in grams of the elements that compose, respectively, the stem, leaves, grain, cob, and roots is shown in the last half of Table V. These values expressed in grams were obtained by multiplying the average weight of the organs by their average percentage composition. The total weight of each of the elements that make up the mature plant is expressed graphically in Figure 1.

TABLE III.—*Elemental composition of the leaves, stems, cobs, and grain of five Pride of Saline corn plants grown in the field at Manhattan, Kans., in 1920*

Plant No.	Organ.	Dry weight.	ELEMENTS.													
			Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorin.	Manganese.
			PERCENTAGE OF THE ELEMENTS, DRY BASIS.													
1	Leaves		41.18	45.28	5.75	1.16	0.169	1.57	0.56	0.25	0.25	0.070	2.44	0.075	0.34	0.031
2	do		40.85	44.49	6.20	1.40	.202	1.57	.38	.23	.23	.073	2.20	.086	.43	.023
3	do		42.25	43.35	5.77	1.43	.225	1.32	.46	.20	.23	.081	2.69	.074	.073	.036
4	do		40.85	44.24	5.82	1.21	.186	1.54	.49	.17	.24	.058	2.57	.071	.154	.044
5	do		41.22	41.95	5.78	1.32	.256	1.38	.47	.20	.23	.070	3.05	.063	.115	.025
1	Stem		43.96	43.91	5.66	.88	.073	1.64	.14	.14	.15	.052	.43	.016	.40	.016
2	do		44.08	42.90	5.83	.94	.093	1.09	.19	.18	.13	.040	.30	.014	.40	.010
3	do		46.35	42.50	6.00	.50	.072	1.12	.14	.12	.16	.046	.41	.012	.13	.023
4	do		44.35	45.00	5.93	.85	.081	1.13	.20	.14	.17	.046	.49	.012	.072	.017
5	do		43.80	45.20	6.08	1.02	.124	1.16	.20	.21	.17	.074	.47	.012	.12	.018
1	Grain		45.25	45.05	7.01	2.14	.31	.37	.035	.20	.12	.049	.018	.020	.026	.027
2	do		44.48	45.42	7.02	2.14	.33	.51	.028	.20	.18	.030	.014	.036	.031	.044
3	do		44.75	45.31	6.93	2.22	.33	.43	.023	.19	.12	.028	.021	.033	.031	.050
4	do		45.10	44.67	6.94	2.24	.40	.45	.021	.19	.14	.055	.016	.014	.031	.043
5	do		44.00	46.04	6.92	2.03	.34	.34	.019	.21	.12	.055	.011	.013	.046	.023
	Cobs		45.75	45.89	6.36	1.38	.094	.46	.022	.11	.021	.025	.133	.052	.12	.031
			TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.													
1	Leaves	209.4	86.23	94.81	12.04	2.42	0.353	3.28	1.17	0.523	0.523	0.146	5.10	0.157	0.711	0.065
2	do	252.5	103.10	112.29	15.64	3.53	.510	3.96	.96	.581	.581	.184	5.55	.217	1.085	.058
3	do	195.8	82.72	84.87	11.29	2.79	.440	2.58	.90	.391	.450	.158	5.27	.144	.142	.070
4	do	235.5	96.20	104.18	13.71	2.84	.438	3.62	1.15	.400	.565	.136	6.05	.167	.362	.104
5	do	279.7	115.29	117.33	16.16	3.69	.716	3.86	1.31	.559	.643	.195	8.53	.176	.391	.069

1	Stems	154.3	67.83	67.75	8.73	1.36	.112	2.53	.21	.210	.231	.080	.66	.024	.617	.024
2	.....do	191.2	84.28	82.02	11.14	1.79	.177	2.08	.36	.344	.248	.076	.57	.026	.760	.019
3	.....do	161.0	74.62	68.42	9.66	.81	.115	1.80	.23	.193	.257	.074	.66	.019	.209	.037
4	.....do	233.0	103.33	104.85	13.81	1.98	.188	2.63	.46	.326	.396	.107	1.14	.027	.167	.039
5	.....do	263.5	115.40	119.10	16.02	2.68	.326	3.05	.52	.553	.447	.194	1.23	.031	.316	.047
1	Grain	320.0	144.80	144.16	23.43	6.84	.990	1.18	.112	.640	.384	.156	.057	.064	.083	.086
2	.....do	272.2	121.07	123.63	19.10	5.82	.890	1.38	.076	.544	.489	.082	.038	.098	.084	.119
3	.....do	232.0	103.82	105.11	16.07	5.15	.760	1.00	.053	.440	.278	.065	.049	.076	.072	.116
4	.....do	212.0	95.61	94.70	14.71	4.74	.850	.96	.045	.406	.299	.117	.034	.029	.066	.092
5	.....do	268.8	118.27	123.75	18.60	5.45	.910	.91	.051	.564	.322	.147	.029	.035	.123	.061
1	Cobs	92.6	42.36	42.49	5.88	1.27	.087	.425	.020	.101	.019	.023	.123	.048	.111	.029
2	.....do	75.3	34.44	34.55	4.78	1.03	.071	.346	.017	.083	.016	.018	.100	.039	.090	.023
3	.....do	70.7	32.34	32.44	4.49	.98	.066	.325	.015	.078	.015	.018	.094	.037	.085	.022
4	.....do	65.3	29.87	29.96	4.15	.90	.061	.30	.014	.072	.014	.016	.086	.034	.078	.020
5	.....do	87.3	39.93	40.06	5.55	1.20	.082	.40	.019	.096	.018	.022	.116	.045	.104	.027

TABLE IV. *Elemental composition of the roots of five Pride of Saline corn plants grown in containers at Manhattan, Kans., in 1920*

Plant.	Dry weight.	ELEMENTS.												
		Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorin.	Manganese.
		PERCENTAGE OF THE ELEMENTS, DRY BASIS.												
A		42.50	46.14	5.93	1.27	0.141	0.27	0.58	0.18	0.28	0.43	6.63	0.89	0.12	0.045
B		43.37	45.80	5.79	1.24	.122	.57	.56	.17	.25	.44	4.89	.94	.09	.088
C		43.35	43.15	6.01	1.27	.121	.56	.64	.18	.23	.45	3.38	.89	.10	.056
D		42.65	42.05	5.56	1.40	.101	.37	.72	.15	.24	.50	4.17	1.15	.12	.089
E		39.70	40.78	5.33	1.16	.113	.65	.56	.16	.23	.76	3.13	1.02	.11	.055
		TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.												
A	59.5	25.28	27.45	3.53	0.75	0.083	0.160	0.345	0.107	0.166	0.255	3.94	0.529	0.071	0.027
B	62.4	27.06	28.57	3.61	.77	.076	.355	.349	.106	.156	.274	3.05	.586	.056	.055
C	60.7	26.31	26.19	3.64	.77	.073	.339	.388	.109	.139	.273	2.05	.540	.061	.034
D	53.9	22.98	22.66	3.00	.75	.054	.199	.388	.081	.129	.269	2.24	.619	.064	.048
E	66.0	26.20	26.91	3.51	.76	.074	.429	.369	.105	.151	.501	2.06	.673	.073	.036

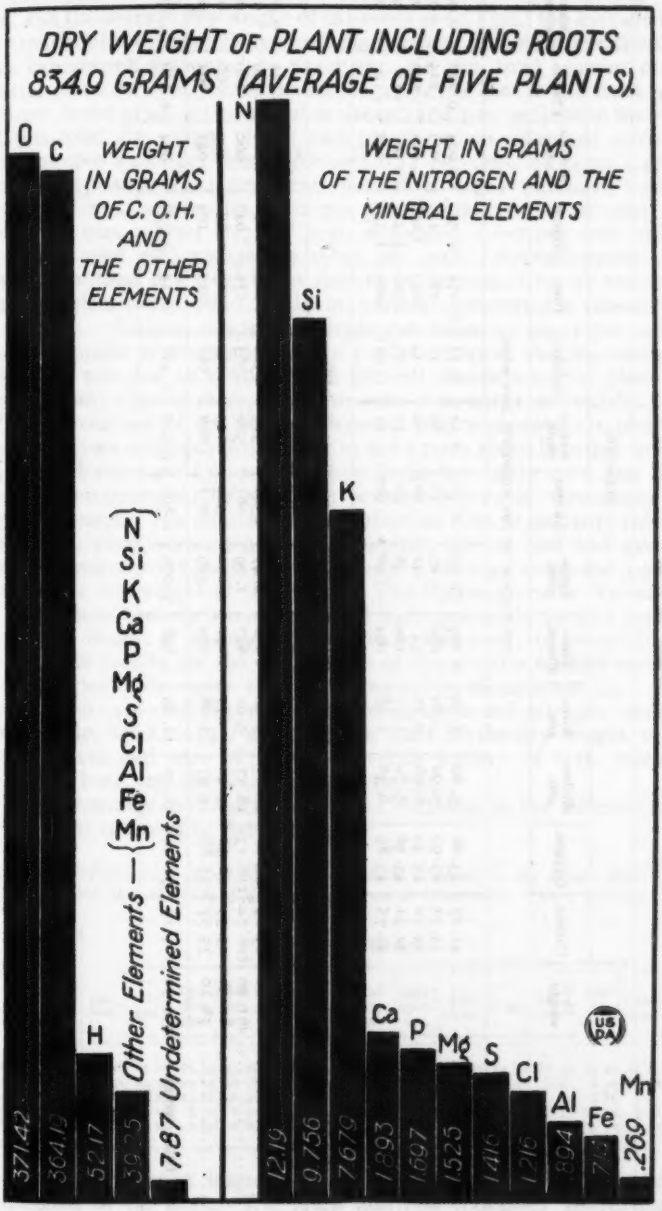

FIG. 1.—Relative amount of the elements that composed the dry matter of Pride of Saline corn grown at Manhattan, Kans., in 1920.

TABLE V.—*Average amount of the elements in the leaves, stems, cobs, grain, and roots of five Pride of Saline corn plants grown at Manhattan, Kans., in 1920*

Organ.	Dry weight.	ELEMENTS. Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorin.	Manganese.
		PERCENTAGE OF THE ELEMENTS, DRY BASIS.													
Leaves		41.27	43.86	5.86	1.30	0.207	1.48	0.47	0.21	0.24	0.070	2.59	0.074	0.222	0.043
Stems		44.51	43.90	5.90	.84	.089	1.23	.17	.16	.16	.052	.42	.013	.224	.017
Grain		44.72	45.30	6.96	2.15	.34	.42	.025	.20	.14	.043	.016	.023	.033	.037
Roots		42.31	43.58	5.72	1.27	.120	.48	.61	.17	.25	.52	4.44	.98	.11	.066
Cobs		45.75	45.89	6.36	1.38	.094	.46	.022	.11	.021	.025	1.33	.052	.12	.031
		TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.													
Leaves	234.60	96.82	102.89	13.74	3.05	0.486	3.47	1.101	0.493	0.563	0.164	6.08	0.174	0.521	0.075
Stems	200.60	89.29	88.06	11.83	1.68	.178	2.47	.341	.321	.321	.104	.84	.026	.449	.034
Grain	261.00	116.72	118.23	18.17	5.61	.887	1.09	.065	.522	.365	.112	.042	.060	.086	.096
Roots	60.50	25.59	26.36	3.46	.77	.072	.290	.369	.103	.151	.315	2.69	.593	.066	.040
Cobs	78.20	35.77	35.88	4.97	1.08	.074	.359	.017	.086	.016	.019	.104	.041	.094	.024
Average total weight	834.90	364.19	371.42	52.17	12.19	1.697	7.679	1.893	1.525	1.416	.714	9.756	.894	1.216	.269

Taking the average dry weight of the aerial parts of the plants as 774.4 gm. and the average dry weight of the roots as 60.5 gm., the average total dry weight of a Pride of Saline corn plant grown under the conditions of this experiment amounted to 834.9 gm. Of this total amount of dry matter 28.10 per cent was in the leaves, 24.02 per cent in the stem, 31.26 per cent in the grain, 9.37 per cent in the cob and 7.25 per cent in the roots. Of this total dry weight of the corn plant, carbon composed 43.62 per cent, oxygen 44.48 per cent, hydrogen 6.24 per cent, nitrogen 1.46 per cent, phosphorus 0.204 per cent, potassium 0.919 per cent, calcium 0.226 per cent, magnesium 0.182 per cent, sulphur 0.169 per cent, iron 0.085 per cent, silicon 1.17 per cent, aluminum 0.107 per cent, chlorin 0.145 per cent and manganese 0.032 per cent. Approximately 1 per cent of the total dry weight was thus to be accounted for by the undetermined elements of which, no doubt, sodium represented a considerable proportion. Carbon, oxygen and hydrogen made up 94.34 per cent of the dry weight of the plant. Since 1.46 per cent of the dry matter of the plant was due to nitrogen, the mineral elements in the plant represented only slightly more than 4 per cent of its entire dry weight.

The percentage of the various elements that composed the plants in these analyses corresponded closely to the figures given by other investigators. Hornberger (*3*) worked with Badischer Early corn and made chemical analyses of the plants at seven-day periods throughout the growing season. His analyses show that at the time of maturity calcium, potassium, phosphorus, magnesium, sulphur, silicon, iron and nitrogen made up, respectively, 0.51, 1.19, 0.27, 0.42, 0.08, 0.40, 0.05, and 1.54 per cent of the dry weight of the plants. The figures given by Vivian (*12, p. 9–11*) show that carbon, oxygen and hydrogen made up 92.7 per cent of the dry weight of the corn plant which was ready to be cut for the shock. His results for the percentages of the various mineral elements were practically the same as those reported by Hornberger (*3*). Jones and Huston (*5*) found that potassium, phosphorus and nitrogen made up, respectively, 0.828, 0.193, and 1.17 per cent of the dry weight of the stems, leaves and ears of Riley's Favorite variety of corn when the grain was hard and the plant ready to be cut.

The percentage distribution of the 14 elements in the different parts of the plant is shown in Table VI.

TABLE VI.—*Percentage distribution of the different elements in the leaves, stem, grain, cob, and roots of Pride of Saline corn grown at Manhattan, Kans., in 1920*

Organ.	Elements.													
	Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorin.	Manganese.
Leaves	26.58	27.70	26.33	25.01	28.63	45.18	58.16	32.32	39.75	22.96	62.32	19.46	42.84	27.88
Stems	24.51	23.70	22.67	13.78	10.48	32.16	18.02	21.04	22.66	14.57	8.61	2.90	36.92	12.64
Grain	32.04	31.83	34.82	46.01	52.26	14.19	3.43	34.22	25.77	15.68	.43	6.71	7.07	35.68
Roots	7.03	7.09	9.52	6.31	4.24	3.76	19.49	6.75	10.66	44.11	27.57	66.33	5.43	14.87
Cobs	9.82	9.66	6.63	8.85	4.36	4.67	.89	5.63	1.12	2.66	1.06	4.58	7.75	8.90

Some of the more important facts shown in this table should be mentioned in the text. The grain and cob contained approximately 55 per cent of the total nitrogen, while 25 per cent of this element occurred in the leaves. The phosphorus was distributed in the same relative

proportion, since 56 per cent of the total phosphorus was in the grain and cob and 29 per cent in the leaves. Approximately 45 per cent of the potassium occurred in the leaves, 32 per cent in the stem and only 19 per cent in the grain and cobs. More than 58 per cent of the calcium was in the leaves, while the stems and roots contained 18 and 19 per cent, respectively. The proportion of calcium in the ear was small and amounted to slightly more than 4 per cent of the total calcium in the plant. In contrast to the calcium, more than 39 per cent of the magnesium occurred in the grain and cob while the proportion of this element in the leaves and stem amounted to 32 and 21 per cent, respectively. The leaves contained 39 per cent of the sulphur, the stem 22 per cent and the grain and cob 26 per cent. Approximately 22 per cent of the iron was found in the leaves, while the stem and grain each contained 15 per cent of this element. The leaves contained the greater part of the silicon, since over 62 per cent of the total amount of this element occurred in them. The ear contained about 1.5 per cent of the silicon and of this amount less than one-half per cent occurred in the grain. The leaves and the stem contained most of the chlorin; 42 per cent of this element occurred in the former and 36 per cent in the latter. Approximately 44 per cent of the manganese occurred in the grain and cob and over 27 per cent in the leaves. The roots contained 6.3 per cent of the nitrogen, 4.2 per cent of the phosphorus, 3.7 per cent of the potassium, 19.5 per cent of the calcium, 6.7 per cent of the magnesium, 10.6 per cent of the sulphur, 44.1 per cent of the iron, 27.5 per cent of the silicon, 66.4 per cent of the aluminum, 5.4 per cent of the chlorin and 14.8 per cent of the manganese. The relatively large proportions of calcium, iron, silicon and aluminum in the roots was due in part, probably, to the minute soil particles that were partially embedded in the surface of the roots and were not removed in the preparation of the material for analyses.

WEIGHT OF THE ELEMENTS REMOVED FROM THE AIR AND SOIL BY AN ACRE CROP OF CORN

Since so many factors can influence the yield and composition of plants, any data concerning the amount of the various elements removed per acre for a given crop are applicable only for the cultural and climatic conditions under which the plants were grown. The recent work of Duley and Miller (*1*) concerning the effect of the supply of nutrients upon the character and composition of the corn plant especially emphasizes this fact. In the experiment herein reported, the plants were grown in 42-inch rows and the plants in the row thinned to a distance of 2 feet. If the stand were perfect under these conditions, there would be 6,270 plants per acre, but in order to allow some leeway 6,200 plants were considered as the stand per acre in estimating the yield of dry matter. The total dry matter produced by 6,200 plants, the average dry weight of which, including the roots, was 834.9 gm., would amount to 11,389 pounds per acre. Since the dry matter of the roots weighed 60.5 gm. per plant, the dry matter produced per acre by them would amount to 825 pounds. The total dry weight of the plants above ground would thus amount to 10,564 pounds per acre. The average dry weight of the lower foot of the stem was determined in order to ascertain the amount of dry matter left on the field by the stubs when the plants were cut at a height of 1 foot from the ground. The average weight of the lower foot of the stem was 28.3 gm. If 6,200 plants were considered as

the stand per acre, the weight of dry matter remaining in the stubs would be 386 pounds. The total weight of dry matter left in the field by the stubs and roots amounted, according to these estimations, to 1,211 pounds per acre, or approximately 10.6 per cent of the total dry matter produced. The total dry matter produced per acre, exclusive of the roots and the stubs 1 foot in height, amounted to 10,178 pounds per acre. This yield of dry matter of the stems, leaves, and ears of the corn plant is considerably higher than that reported by other investigators, but it is difficult to make comparisons since in some cases the variety of corn grown and the height of the stalks when cut at harvesting were not stated. Ladd (*6*) reported a yield of 7,918 pounds of dry matter per acre for the King Phillip variety at the Geneva (N. Y.) Agricultural Experiment Station, but did not mention how much of the stalk was left on the stub. Smith (*11*) obtained a yield of 8,020 pounds of dry matter per acre at the Michigan Experiment Station, but no record of the variety used or the method of harvesting is given. Schweitzer (*10*) at the Missouri Experiment Station considered 6,528 plants to the acre and estimated the total yield of dry matter at 7,892 pounds, but neglected to state the variety of corn used. Jones and Huston (*5*) estimated the yield of dry matter of Riley's Favorite grown at the Indiana Experiment Station at 9,412 pounds when the plants were harvested at the surface of the ground and when 10,000 plants were considered as the stand per acre. Ince (*4*) states that the average yield of dry matter of the ears, stems, and leaves of numerous varieties of corn grown at the North Dakota Experiment Station was 6,130 pounds, but he does not mention how much of the stalk was left on the ground in harvesting.

The estimated weight in pounds of the elements removed per acre from the soil and the air by the entire corn plant and by each of the several organs or parts is shown in Table VII.

TABLE VII.—*Estimated weight in pounds of the elements removed per acre from the air and soil by 6,200 Pride of Saline corn plants grown at Manhattan, Kans., in 1920*

Element.	Weight in pounds of the elements in—									
	Entire plant.	Roots.	Aerial parts.	Stubs 1 foot high.	Roots and stubs 1 foot high.	Grain.	Cobs.	Cobs and grain.	Leaves.	Stems.
Carbon	4,967.55	349.04	4,618.51	171.59	520.63	1,592.06	487.90	2,079.96	1,320.62	1,217.91
Oxygen	5,066.17	359.55	4,706.62	169.27	528.82	1,612.65	489.40	2,102.05	1,403.41	1,201.13
Hydrogen	711.60	47.19	664.41	22.64	69.83	247.83	67.79	315.62	187.41	161.36
Nitrogen	166.27	10.50	155.77	3.22	13.72	76.52	14.73	91.25	41.60	22.91
Phosphorus	23.15	.98	22.17	.04	1.02	12.09	1.01	13.10	6.63	2.42
Potassium	104.74	3.95	100.79	4.74	8.69	14.86	4.89	19.75	47.33	33.69
Calcium	25.82	5.03	20.79	.65	5.68	.89	.23	1.12	15.02	4.65
Magnesium	20.80	1.40	19.40	.61	2.01	7.12	1.17	8.29	6.72	4.37
Sulphur	19.31	2.06	17.25	.61	2.67	4.98	.22	5.20	7.68	4.37
Iron	9.74	4.30	5.44	.19	4.49	1.52	.26	1.78	2.23	1.41
Silicon	133.07	36.70	96.37	1.61	38.31	.42	1.42	1.84	82.93	11.45
Aluminum	12.19	8.09	4.00	.05	8.14	.81	.56	1.37	2.37	.35
Chlorin	16.59	.90	15.69	.84	1.74	1.17	1.28	2.45	7.11	6.12
Manganese	3.67	.35	3.12	.07	.62	1.30	.32	1.62	1.02	.46

These results were obtained by multiplying the weight of each of the different elements in the different organs, as shown in Table V, by 6,200 and reducing the results thus obtained to pounds. The results are self-explanatory and need not be discussed in detail in the text. The figures

in regard to those elements which have been determined by other investigators should be mentioned, however, for the purpose of comparison. The estimated amount of phosphorus, calcium, magnesium, potassium, silicon, and nitrogen removed per acre by the aerial parts of a Pride of Saline corn plant was respectively 22.17, 20.79, 19.40, 100.79, 96.37 and 155.77 pounds. Schweitzer (*10*) considered 6,528 plants of corn per acre and estimated that 83 pounds of phosphorus, 8.9 pounds of calcium, 15 pounds of magnesium, 50 pounds of potassium, 43 pounds of silicon and 135 pounds of nitrogen were removed from the soil by the stems, leaves, and ears of these plants. Hopkins (*2, p. 154*) estimated that a corn crop producing 100 bushels of grain and three tons of stover per acre would remove from the soil 148 pounds of nitrogen, 23 pounds of phosphorus, 71 pounds of potassium, 17 pounds of magnesium, 22.3 pounds of calcium, 5.2 pounds of iron and 6 pounds of sulphur. Jones and Huston (*5*) estimated that the aerial parts of Riley's Favorite corn removed, per acre, 18.3 pounds of phosphorus, 76.2 pounds of potassium and 110.6 pounds of nitrogen. The estimated weight of these elements removed per acre by a crop of Pride of Saline corn grown under the conditions of this experiment was considerably higher than that reported by Schweitzer (*10*) and Jones and Huston (*5*). The greater yield of dry matter per acre as estimated from our experiment would account for a considerable portion of the differences, since in some cases, at least, the percentage composition is approximately the same as that reported by these investigators. Our estimations compare very closely with those of Hopkins (*2, p. 75*) with the exceptions of the amounts of potassium and sulphur removed by the crop. The relatively large amount of potassium in the plants grown at Manhattan, Kans., was due probably to the fact that the soil in which the plants were grown was especially rich in this element, while the small amount of sulphur obtained by the earlier investigators was due to the naccuracy of the methods used in its estimation.

SUMMARY

An elemental analysis was made of five Pride of Saline corn plants grown in the field at Manhattan, Kans., in 1920. Determinations were made for carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, silicon, aluminum, chlorin and manganese.

In order to determine the variation in the composition of the different plants and their organs, separate analyses were made of the leaves, stem and grain of each of the five plants. A separate analysis was also made of each of five root systems of plants that were grown in soil in large metal containers.

The analytical results are tabulated and discussed in the text under the following headings:

Variations in the percentage and in the total weight of the elements that composed the various organs of the plants.

The percentage distribution of the different elements in the leaves, stems, grain, cobs, and roots of the plants.

The number of pounds of each of the elements removed per acre from the soil and air by a crop of corn.

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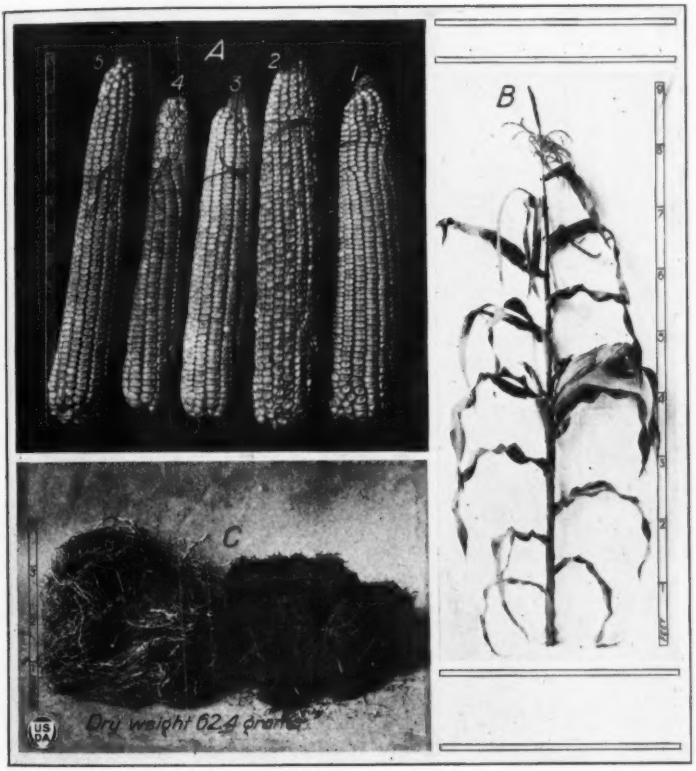
PLATE 1

A.—Ears of the five Pride of Saline corn plants that were used for the elemental analyses.

B.—Pride of Saline corn plant No. 4 showing the general appearance of the five plants used for the elemental analyses.

C.—General appearance of one (B, Table IV) of the five root systems of Pride of Saline corn plants used in the chemical analyses.


A
5
4
3
2
1
B
C
Dry weight 62.4 grams

WHEAT SCAB AND CORN ROOTROT CAUSED BY GIBBERELLA SAUBINETII IN RELATION TO CROP SUCCESSIONS[1]

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INTRODUCTION

It is a well-known fact that corn, like other field crops, can not be grown year after year on the same soil without harmful effects. Winter wheat is a crop well adapted to the corn belt. Like corn, it is one of the most desirable crops from the standpoint of immediate financial returns. It, therefore, has become a common practice in the Corn Belt to include both corn and wheat in the rotation. In many cases wheat is sown directly after corn.

In preparing corn land for wheat several cultural methods are in common use, but very seldom is the field plowed before sowing the wheat. Winter wheat is often sown with a one-horse drill between the standing rows of corn in the early fall. After the ground has become frozen the corn is husked and the cornstalks are broken down by dragging a pole over them. In some localities the corn is husked early, the cornstalks broken down and cut up with a disk harrow, and the field sown with winter wheat in the fall or spring wheat in the early spring. In other sections the corn is cut and shocked, after which wheat is sown in the disked corn stubble. Another practice in rather general use is to cut the corn for silage and thus remove all of it, except the stubble, from the field before sowing the wheat. The wheat is commonly followed by a crop of oats, after which corn again is planted, or the wheat may be followed directly by corn. Although wheat is generally sown directly after corn, yet the crop sequence often is oats after corn and wheat after the oats. As will be pointed out later, this is a much more desirable crop sequence from the standpoint of the control of wheat scab (Fusarium blight).

In some regions a legume, such as clover or soybeans, frequently is included in the rotation. In sections where the farming has become more intensive or where the soils are less fertile, clover is sown every three or four years. This not only builds up the nitrogen content of the soil but tends to reduce the accumulation of soil-borne parasites.

[1] Received for publication Jan. 29, 1924. The investigations upon which this paper is based were conducted as a cooperative project between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company, Bloomington, Ill.

Journal of Agricultural Research, Washington, D. C.

Vol. XXVII, No. 11
Mar. 15, 1924
Key No. G-373

85606—24——5

WHEAT SCAB IN RELATION TO PREVIOUS CROPPING

REVIEW OF LITERATURE

Latta, Arthur, and Huston (*15*),[2] in 1891, mentioned the prevalence of scab on a plat where wheat had been grown continuously and on an adjacent plat where wheat and corn had been grown alternately for 11 years. This seems to be the first recorded observation on the relation of previous cropping to the occurrence of wheat scab. In 1909, Selby and Mánns (*19*) also recorded observations on the relation of previous cropping to the production of wheat scab. They noticed that on the experimental plats that had been sown to wheat continuously for a number of years scab was worse than on the plats where the crops had been rotated. Some years later Bolley (*3*) emphasized the importance of rotating wheat with some other unrelated crop to keep down wheat scab. He recommended rotating with clover, alfalfa, grasses, potatoes, flax, and corn.

In 1918, Hoffer, Johnson, and Atanasoff (*8*) reported that there was a greater abundance of wheat scab in fields where wheat was grown immediately following corn that was infected with the Fusarium root and stalk rots. They found that wheat-scab infection may take place from Gibberella spores growing on old diseased cornstalks and that corn rootrot can be produced under laboratory conditions by Gibberella spores isolated from scabbed wheat. During the same year Hoffer and Holbert (*7*) reported a greater amount of scab when wheat was sown in infested corn fields.

In 1919, Holbert, Trost, and Hoffer (*11*) reported on the occurrence of wheat scab as affected by crop rotations. Their observations, which covered 28 fields, comprising 1,500 acres, confirmed previous observations that wheat following corn is scabbed more severely than that following any other crop.

Since then, reports have appeared by Johnson and Haskell (*14, p. 24–26*), Johnson and Dickson (*13*), Fromme (*6, p. 141*), and Adams (*1*) stating that wheat-scab infection is heaviest when wheat follows a corn crop.

SURVEY OF 1919[3]

In 1919 counts were made of wheat-scab infection following various common crops in seven different States. There was an unusual epidemic of scab during this season. Most of the wheat examined was in the hard-dough stage and some of it was ripe. Each percentage determination was made by counting a hundred heads along a drill row and breaking off all heads that showed the presence of scab. The number of scabbed heads picked off then gave the percentage of infected heads. At the close of each day, the heads thus collected were sent to the laboratory at Madison, Wis., for identification of the organisms. In making the scab counts, four or more, and often over a hundred, percentage determinations were made in different parts of each field.

In Wisconsin, where the grain had recently been cut, the percentage determinations were made from entire bundles. From four to nine bundles were selected from different parts of each field, each bundle containing over 2,000 heads.

[2] Reference is made by number (italic) to "Literature cited," pp. 878-879.

[3] L. E. Compton, Junior Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, assisted in obtaining these survey data.

This survey was made primarily for accurate information on the distribution of wheat-scab, but notes were made also on a number of phases of the problem including the crop successions during the three previous years. As the same crop rotation usually is not followed on the different farms in any community, adjacent fields or fields close to each other were observed where wheat followed corn in some cases and in other cases followed other crops.

Wherever wheat followed corn in the various States, perithecia of *Gibberella saubinetii* (Mont.) Sacc. were found in abundance on the old cornstalks and stubble left in the field. To make certain of their identity, specimens were sent to the laboratory at Madison, Wis., from each farm where observations were made.

Table I gives a summary of the data collected in 1919. The difference between the percentage of heads scabbed when wheat followed corn as compared with the percentage when wheat followed some other crop is large enough in each State to be significant. A large number of wheat varieties are included in this summary. There is some varietal difference in the degree of susceptibility to scab, but the differences in respect to previous cropping are similar in all varieties and therefore all are summarized in one table.

Scab also affects other cereals, including rye and oats, but generally the infection is of less extent than in wheat. Table I shows not only that wheat-scab infection is most severe when wheat follows corn, but that it also is greater when wheat follows wheat, rye, or oats than when it follows clover or timothy. The last two crops usually are not attacked by *Gibberella saubinetii*, the chief causal organism of scab of cereals, although Selby and Manns (*19*) report a stem rot of clover produced probably by this parasite. Therefore, the disease is not so likely to be carried over from the previous year on the refuse from these two crops as on refuse from oats, wheat, and corn. These data show that, over a large area, wheat scab occurs most abundantly where wheat follows corn.

TABLE I.—*Average percentages of wheat scab (head blight) in 1919 following different crops in seven States*

State.	Number of counties surveyed.	Number of fields surveyed.	Average percentages of wheat scab after previous crop of—					
			Corn.	Wheat.	Rye.	Oats.	Clover.	Timothy.
Illinois	2	23	59	33	.....	33	22	.....
Indiana	10	47	39	25	30	16	20	.....
Iowa	9	27	71	49	.....	30	.....	.....
Minnesota	4	20	66	58	50	45	.....	5
Ohio	5	39	33	6	.....	11	1	17
Tennessee	5	16	21	4	1	.....	9	.....
Wisconsin	4	10	14	.....	.....	1	5	3
Average	.....	.....	43.3	29.2	27.0	22.7	11.4	8.3

SURVEY OF 1920

The survey of 1920, as summarized in Table II, was confined to McLean County, Ill. All of the fields surveyed were on the same type of soil, in a more or less contiguous area, and all were sown with Turkey

wheat. While only a small wheat acreage was surveyed as compared with that of the previous year, the conditions under which the crop was grown were very much more uniform, so that in many ways the data are more significant than those obtained in 1919.

TABLE II.—*Percentages of scab (head blight) in Turkey wheat following different crops, in McLean County, Ill., in 1920*

Field Number.	Size of field.	Previous crop.	Percentages of scab at different locations in fields.												Grand average following each crop.
			1	2	3	4	5	6	7	8	9	10	11	12	Average.	
	Acres.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
107	80	Corn	6.0	10.0	4.0	5.0	2.0	3.0	6.0	5.0	.....	.....	.....	.....	5.1	
479	30	.....do.....	8.0	3.0	3.0	10.0	2.0	2.0	5.0	.....	.....	.....	.....	.....	4.7	
473	10	.....do.....	2.0	4.0	2.0	4.0	.....	.....	.....	.....	.....	.....	.....	.....	3.0	
475	10	.....do.....	3.0	3.0	5.0	3.0	5.0	3.0	.....	.....	.....	.....	.....	.....	3.7	4.6
472	10	.....do.....	3.0	4.0	4.0	5.0	3.0	3.0	.....	.....	.....	.....	.....	.....	3.7	
660	40	.....do.....	5.0	11.0	9.0	9.0	4.0	10.0	8.0	7.0	.....	.....	.....	.....	7.9	
167	60	.....do.....	5.0	9.0	4.0	3.0	7.0	2.0	.....	.....	.....	.....	.....	.....	5.0	
600	33	.....do.....	7.0	1.0	3.0	2.0	4.0	.....	.....	.....	.....	.....	.....	.....	3.4	
43	100	Wheat.....	6.0	2.0	1.0	3.0	1.0	1.0	1.0	.0	.2	.2	.2	1.0	1.4	
128	40	.....do.....	.2	.5	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.3	
108	18	.....do.....	.0	.5	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	
400	40	.....do.....	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	
42	60	.....do.....	1.0	2.0	1.0	2.0	3.0	2.0	.5	.2	.2	.5	.....	.....	1.2	1.0
47	75	.....do.....	.2	.5	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.3	
123	50	.....do.....	2.0	1.0	.2	.2	1.0	.....	.....	.....	.....	.....	.....	.....	.9	
674	60	.....do.....	2.0	1.0	3.0	4.0	3.0	2.0	3.0	1.0	.....	.....	.....	.....	2.4	
496	80	.....do.....	2.0	2.0	1.0	1.0	3.0	1.0	.....	.....	.....	.....	.....	.....	1.7	
127	90	Oats.....	.5	.2	.5	.5	2.0	5.0	4.0	2.0	5.0	4.0	4.0	2.0	2.5	
125	20	.....do.....	.2	.2	.0	.2	.2	.....	.....	.....	.....	.....	.....	.....	.2	1.7
190	60	.....do.....	.5	.2	2.0	.2	1.0	.5	.....	.....	.....	.....	.....	.....	.7	
697	35	.....do.....	3.0	3.0	4.0	4.0	3.0	3.0	.....	.....	.....	.....	.....	.....	3.3	
400	40	Timothy...	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	.2

While the wheat-scab infection during this season was generally light, there was enough to show a striking difference in the percentage of scab resulting where wheat followed corn and where wheat followed some other crop. In every field which had produced a corn crop during the previous year the percentage of wheat scab was much higher than where some other crop had preceded wheat, the average of scab infection being more than 170 per cent higher than in any other crop sequence.

The seasonal difference in the percentage of scab was due undoubtedly to weather conditions during the flowering and maturing of the wheat crop. It has been previously observed by Schmitz (*18*), Mortensen (*16*), Beckwith (*2*), Schaffnit (*17*), and others, that there is a greater abundance of wheat scab during moist seasons. Indications are that moisture was the chief factor in producing the seasonal differences in infection during these two years, as the weather during and following flowering in 1919 was very moist, whereas in 1920 the entire period was dry. Schaffnit (*17*), working with Fusarium organisms isolated from scabbed cereals, has shown that a relative humidity of not less than 55 to 60 per cent is necessary for their growth and that the substratum must contain 20 to 30 per cent of moisture.

STUDIES IN 1921

In 1921, an effort was made to secure data under even more uniform conditions than in the previous years. After considerable scouting, four

pairs of fields were located, each pair consisting of two adjacent wheat fields, on the same type of soil of about the same fertility, with similar topography and drainage, and sown at nearly the same time with Turkey wheat. One field of each pair had been planted to corn the previous year and the other had been cropped with either wheat or oats. Numerous counts were made in each field, as shown in Table III. Some variation in the percentage of scabbed heads occurred in each field. General observations in previous years indicated that scab infection generally was most severe at the lower field elevations. This was probably caused by the more succulent growth in these locations. Climatic conditions in 1921 were such that the growth in the low areas was not very succulent. On the contrary, the crop was thin and poorly developed in many of these lower places. An attempt was made to cover all of the different conditions in each field so as to establish correct averages. In every case the land was only gently rolling and the differences in elevation were not great. Table III shows that in this year the percentage of wheat scab was only slightly affected by differences in elevation, the greater infection, if any, occurring on the higher ground where, in this season, the wheat had made the best growth.

TABLE III.—*Percentages of scab in Turkey wheat at different elevations in four pairs of adjacent fields (A, B, C, D), on brown silt loam with similar fertility, drainage, and topography, but having had different previous crops, in McLean County, Ill., in 1921*

Group.	Field number.	Size of field.	Previous crop.	Elevation.	Percentage scab at different locations in fields.									Field average.
					1	2	3	4	5	6	7	8	Average.	
		Acres.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
A	424	40	Corn	High	40	31	43	43	.....	.....	.....	.....	39.3	.....
				Medium	53	52	14	26	39	41	.....	.....	37.5	.....
				Low	29	26	9	19	15	25	.....	.....	20.5	31.6
	428	60	Wheat	High	4	5	2	5	3	1	5	6	3.9	.....
				Medium	3	4	3	7	.....	.....	.....	.....	4.3	.....
				Low	4	4	2	2	3	6	.....	.....	3.5	3.8
B	610	20	Corn	High	9	16	13	26	.....	.....	.....	.....	16.0	.....
				Medium	19	16	14	10	.....	.....	.....	.....	14.8	.....
				Low	13	13	8	7	.....	.....	.....	.....	10.3	13.7
	611	20	Wheat	High	1	4	4	4	.....	.....	.....	.....	3.3	.....
				Medium	4	5	2	4	.....	.....	.....	.....	3.8	.....
				Low	2	6	3	4	.....	.....	.....	.....	3.8	3.6
C	133	80	Corn	High	22	14	12	18	.....	.....	.....	.....	16.5	.....
				Medium	22	22	14	12	18	14	.....	.....	17.0	.....
				Low	6	8	10	12	16	14	.....	.....	11.0	14.6
	132	80	Oats	High	8	11	8	.....	.....	.....	.....	.....	9.0	.....
				Medium	3	6	8	.....	.....	.....	.....	.....	5.7	.....
				Low	6	6	8	4	.....	.....	.....	.....	6.0	6.8
D	109	20	Corn	Medium	8	4	22	18	9	6	7	11	10.6	.....
				Low	11	13	5	3	.....	.....	.....	.....	8.0	9.8
	107	40	Oats	High	3	5	3	3	.....	.....	.....	.....	3.5	.....
				Medium	3	4	5	4	.....	.....	.....	.....	4.0	.....
				Low	5	5	1	5	.....	.....	.....	.....	4.0	3.8

In group A the wheat was drilled in a field on which the corn was still standing. This left all the old cornstalks in the wheat field. In the other groups, the corn had been removed for silage, leaving only the corn stubble and a very few broken or scattered stalks that were not picked up.

Table III shows that, as in the previous year, scab infections were more than a hundred per cent greater where wheat followed corn than where wheat followed other crops. It also shows that infection is much worse when all the cornstalks are left on the field than when nearly all of them are removed. It must be borne in mind, however, that the corn stubbles were left exposed and that they were thus an important factor in increasing the disease in fields 610, 133, and 109, as compared with fields 611, 132, and 107, respectively.

The study of the influence of the previous crop on the development of wheat scab had shown that the abundant development of perithecia on the old cornstalks determined to a large measure the relative amount of wheat scab. It was important, therefore, to determine the extent to which these perithecia would develop on stalks of relatively resistant strains of corn. Such strains of corn were available, as considerable work had been done by J. R. Holbert and his associates (*10*, *12* [4]) in the breeding and selection of strains resistant to the root and stalk rots. In the fall of 1920 an experiment was planned with this corn to determine the extent to which the perithecia of Gibberella would develop on stalks from corn showing considerable resistance to the root- and stalk-rots. Stalks were obtained from three different selections: (1) The most disease-resistant obtainable; (2) comparatively disease-resistant; and (3) disease-susceptible. A large bundle of each kind was taken to a wheat field in the fall after the wheat had been sown and each bundle was scattered sufficiently to insure that each stalk came in contact with the soil. Just prior to wheat harvest the following year the cornstalks were collected and taken to the laboratory to determine the relative abundance of Gibberella perithecia on those from each selection. Estimates were made on representative portions from each stalk, each portion including a node and an internode. To establish standards, five pieces were selected with perithecia ranging in abundance from few to very numerous. On the piece where the perithecia were very numerous they covered about half the surface. Therefore, this sample was classed as 50 per cent covered. The piece having only a few perithecia was rated as 10 per cent, as this was about the proportion of surface covered. The other three portions were intermediate, differing by about 10 per cent, thus making the series 10, 20, 30, 40, and 50 per cent covered. These samples were used as standards in estimating the percentages of surface covered on all the portions of each stalk. These percentages were then averaged to get the average percentage for each stalk. The results are given in Table IV.

It will be noticed that there were not so many perithecia on stalks of the resistant selections. Nevertheless there were sufficient perithecia even on these to cause an epidemic of wheat scab under favorable conditions. These corn plants were resistant to fungus invasion during their active growing and maturing period, but after maturity and death of the tissues any corn plant is readily invaded by many soil-borne, facultative parasites, and among these *Gibberella saubinetii* is very common. It seems evident, therefore, that growing only disease-resistant corn will not aid materially in avoiding higher percentages of scab infection when wheat follows a crop of corn.

[4] HOLBERT, J. R., and others. PHYSICAL CHARACTERS OF THE CORN EAR IN RELATION TO DISEASE RESISTANCE AND YIELD. Ill. Agr. Exp. Sta. (In press.)

TABLE IV.—*Percentages of stalk surface covered with perithecia of Gibberella saubinetii on old stalks of Yellow Dent corn in strains highly resistant, comparatively resistant, and susceptible to corn rootrot. Data obtained at Bloomington, Ill., in 1921*

Stalk No.	Percentages of surface covered with Gibberella perithecia.		
	Most resistant selection.	Comparatively resistant.	Susceptible.
1	6.2	5.0	14.5
2	3.7	2.8	9.5
3	2.0	.7	19.2
4	0	1.7	6.7
5	7.5	3.3	11.5
6	6.5	4.3	5.0
7	4.4	5.7	5.5
8	2.2	3.5	5.7
9	2.8	5.8	8.6
10	4.4	4.4	4.4
11	1.7	2.9	4.5
12	4.1	7.0	13.2
13	0	2.5	11.0
14	1.5	15.0	5.5
15	2.5	1.0	8.6
16	.....	3.9	13.5
17	.....	1.5	10.5
18	.....	4.5	6.0
19	.....	2.2	5.2
20	.....	10.9	5.7
21	.....	2.5	12.2
Average	3.3	4.3	8.9

SUMMARY OF THE THREE YEARS' DATA

The three years' data as summarized in Table V, and presented graphically in Figure 1, give the average percentages of scab under three different seasonal conditions, that is, a high amount of scab in 1919, a low amount in 1920, and a medium amount in 1921. These seasonal differences are undoubtedly due to weather conditions and can not be controlled, but the relative abundance of scab each year, whether large or small, varies with the previous cropping, and is within the control of the farmer. Whether the average percentage of infection from season to season is large or small, the farmer who sows wheat after corn can easily reduce the infection 50 per cent by using a different crop sequence in his rotations.

TABLE V.—*Average percentages of scab on wheat following various crops in seven States in 1919, and in McLean County, Ill., in 1920 and 1921, being a summary of Tables I, III, and IV*

Year.	Average percentage of wheat-scab infection after previous crop of—					
	Corn.	Wheat.	Rye.	Oats.	Clover.	Timothy.
1919	43.3	29.2	27.0	22.7	11.4	8.3
1920	4.6	1.0	.....	1.7	.....	.2
1921	17.4	3.7	.....	5.3	.....	.....

Corn refuse left from a previous crop generally bears an abundance of perithecia of *Gibberella saubinetii*. Morphological examination and cross inoculations have proved that this is the same organism as that found on scabbed wheat heads. Even though the cornstalks are removed from a field, the remaining corn stubble may show a large number of perithecia (Pl. 1, B, C), unless they are turned under in plowing, and it is very difficult to turn them all under (Pl. 1, A). *Gibberella saubinetii* perithecia also have been found on old straw of wheat, rye, and oats left on the field (Pl. 1, D). However, not so much infection arises from the ascospores from perithecia on wheat, rye, and oat straw because usually the fields are plowed after these crops are grown and

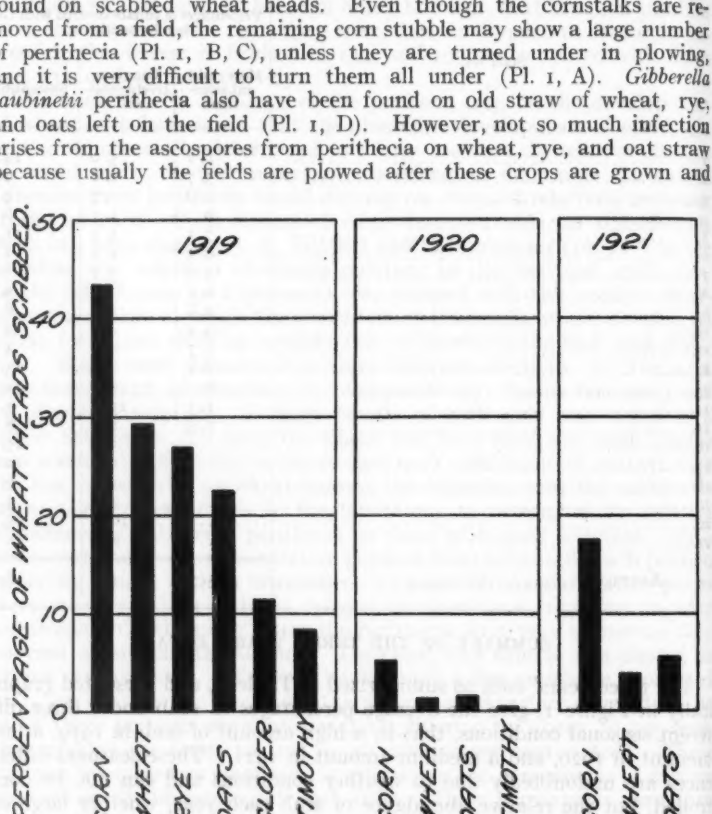

FIG. 1.—Graph showing average percentages of wheat scab following various crops in 1919, 1920, and 1921. Data summarized in Table V.

as a result most of the refuse is turned under. Although the greatest amount of initial infection probably is caused by overwintering ascospores, or conidia formed by the ascospores germinating on the moist soil and crop refuse, the mycelium of the organism also may remain viable on crop refuse and produce abundant conidia during the following spring and summer. Corn refuse bears the greatest amount of perithecia because it apparently is a better medium for growth and holds the moisture better. Therefore, it is of utmost importance to cut the cornstalks close to the ground and remove them from the field if wheat is to be seeded on the land. If winter wheat must be sown after corn, usually it is not practicable to plow the ground; but, in winter-wheat sections especially, if spring wheat is to be sown, such land should be fall plowed in order to bury, as far as possible, all corn refuse. In all

cases where practicable, however, sowing wheat after corn should be avoided.

The data discussed pertain solely to scab or head blight, as only this phase of the disease can be seen and accurately estimated. No account has been taken of seedling blight and weak plants due to the parasite in the soil attacking wheat seedlings. Investigations by Dickson (*4*) and Johnson and Dickson (*13*) show the importance of seedling blight due to soil infestation with *Gibberella saubinetii*. Therefore, the economic importance of not bringing corn and wheat together in a rotation is much greater than data on wheat scab (head blight) alone would indicate. However, it is nearly impossible to get an accurate estimate on seedling blight in a commercial field, and, therefore, this phase has not been considered in this paper.

CORN ROOTROT IN RELATION TO WHEAT-SCAB

The losses resulting from corn rootrot caused by *Gibberella saubinetii* can not be estimated as easily as can those resulting from wheat scab. It can be done, however, more advantageously on carefully controlled experimental plats than by an extensive survey of commercial fields. Fortunately, corn disease investigations have progressed sufficiently (*12*, [5]) so that small quantities of corn that are highly resistant to rootrot under central Illinois conditions can be obtained. This resistant corn was used as a check on the ordinary susceptible corn in all the experiments reported below.

Comparative yields were considered the best index to the extent of rootrot. Extensive data by Holbert et al. (*12*, [5]) show that yields are inversely correlated with the amount of disease found on the germinator and with corn rot produced by pure-culture inoculations. The latter is also shown in Table X. The disease-resistant and disease-susceptible selections, between which comparisons were made, were both selected from the same strain of Yellow Dent corn according to the methods described by Holbert et al.[5] The yields of two fields planted with the same kind of corn but with different previous crops can not be compared directly because there doubtless would be a difference in soil fertility. But by comparing the yield from disease-susceptible corn with the yield from disease-resistant corn, in different crop sequences, a fairly accurate determination can be made. The experimental series reported below were conducted on adjacent fields in each case, and on similar soil of the same elevation and drainage.

In 1920 an experiment was conducted to compare (1) the yields of corn grown on plats which had been cropped to clover during the two previous years and to oats in 1917 and (2) the yields from adjacent plats which had a crop of badly scabbed spring wheat on them in 1919, corn in 1918, and oats in 1917. A diagram of the plats is given in figure 2. Each plat was 4 rows wide and 10 hills long. The hills were 42 inches apart each way and were planted by hand at the rate of 3 kernels per hill. The data as given in Tables VI and VII were obtained by omitting the outside hills of each plat. Thus each plat contained 2 rows of 8 hills each, on which data were taken, a total of 16 hills with a perfect stand of 48 plants.

As shown in Tables VI and VII, the yields from disease-resistant corn were very different on the two series of plats with different previous

[5] HOLBERT, J. R., and others. OP. CIT.

crop successions. There was a reduction in yield of 11.6 bushels, or 13.9 per cent, on the plats where corn followed scabbed wheat. Doubtless this was due largely to differences in fertility; but no doubt a small part was due also to seedling blight and corn rootrot from the previous crops, since the resistant corn was not immune, as is shown by the inoculation experiments with pure cultures on virgin soil, the results of which are given in Table X. The yields from disease-susceptible corn, on the other hand, showed much greater variation in yield due to the previous crop. There was a reduction of 18.5 bushels, or 25.4 per cent, on the

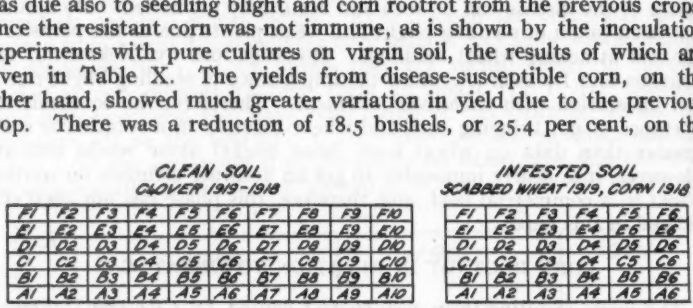
CLEAN SOIL
CLOVER 1919-1918
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10
INFESTED SOIL
SCABBED WHEAT 1919, CORN 1918
F1 F2 F3 F4 F5 F6
E1 E2 E3 E4 E5 E6
D1 D2 D3 D4 D5 D6
C1 C2 C3 C4 C5 C6
B1 B2 B3 B4 B5 B6
A1 A2 A3 A4 A5 A6

FIG. 2.—Diagram of experimental plats in 1920, data on which are presented in Tables VI and VII. Plats A, C, and E, disease-resistant corn; B, D, and F, disease-susceptible corn.

plats previously cropped with wheat. All evidence indicates that the difference between the 13.9 per cent reduction in yield of resistant corn and the 25.4 per cent reduction of susceptible corn was caused by disease-producing organisms in the soil which produced seedling blight, thus reducing the stand, as well as reducing the yield of the remaining plants. In reality, the loss from disease probably was even a little larger than this, because, as already mentioned, part of the 13.9 per cent reduction in yield from the resistant corn was most likely also caused by disease.

The experimental series of 1921, the results of which are given in Tables VIII and IX, were conducted on virgin bluegrass sod and on an

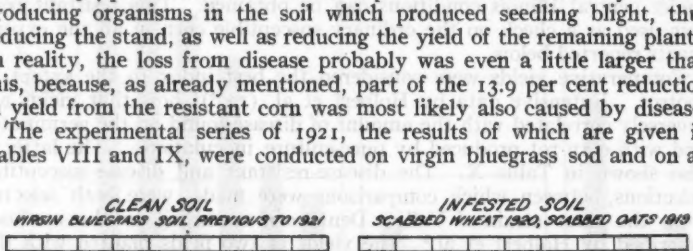
CLEAN SOIL
VIRGIN BLUEGRASS SOIL PREVIOUS TO 1921
F1 F2 F3 F4 F5 F6 F7 F8 F9
E1 E2 E3 E4 E5 E6 E7 E8 E9
D1 D2 D3 D4 D5 D6 D7 D8 D9
C1 C2 C3 C4 C5 C6 C7 C8 C9
B1 B2 B3 B4 B5 B6 B7 B8 B9
A1 A2 A3 A4 A5 A6 A7 A8 A9
INFESTED SOIL
SCABBED WHEAT 1920, SCABBED OATS 1919
F1 F2 F3 F4 F5 F6 F7 F8 F9
E1 E2 E3 E4 E5 E6 E7 E8 E9
D1 D2 D3 D4 D5 D6 D7 D8 D9
C1 C2 C3 C4 C5 C6 C7 C8 C9
B1 B2 B3 B4 B5 B6 B7 B8 B9
A1 A2 A3 A4 A5 A6 A7 A8 A9

FIG. 3.—Diagram of experimental plats in 1921, data on which are presented in Tables VIII and IX. Plats A, C, and E, disease-resistant corn; B, D, and F, disease-susceptible corn; intervening and marginal plats all planted with nearly disease-free seed corn of the same variety.

adjacent field that had grown a crop of moderately scabbed winter wheat during 1920, badly scabbed oats in 1919, and corn during the previous 6 years. Bone meal had been applied to this field so that the productiveness was nearly equal to that of the virgin soil. The plats, figure 3, were of the same size and planted in the same way as those in 1920, but they were arranged differently. Between each two plats used in this experiment was a plat of corn of the same size grown from nearly disease-free seed. Thus the disease-resistant and disease-susceptible corn were bordered by the same kind of corn and apparently should have had the same opportunities. In this series all of the 40 hills in each plat were included in the stand count (Table VIII) and were har-

vested. The acre yield of the disease-resistant corn was 2.7 bushels, or 4.8 per cent, less on the field in which the previous crops of wheat, oats, and corn had been grown, than on the virgin sod, while the acre yield of the disease-susceptible corn was 9.4 bushels, or 17.8 per cent, less. The difference in yield of 13 per cent, then, was due very likely to disease-producing organisms in the soil. But here again, as previously explained, the actual difference probably was a little larger on account of the fact that some reduction in yield of the resistant corn also was chargeable to the attacks of *Gibberella saubinetii*. These data clearly indicate that ordinary susceptible dent corn may suffer a significant reduction in yield when grown after a crop of badly scabbed wheat.

CORN INOCULATIONS WITH GIBBERELLA SAUBINETII

THE ORGANISM

During the season of 1919, over 2,000 scabbed wheat heads collected in the survey in the six States included in the rotation studies mentioned, as well as from seven other States, were sent to the laboratory at Madison, Wis., for identification of the organisms. As reported by Dickson, Johann, and Wineland (*5*), 94 per cent of the scab was found to be caused by *Gibberella saubinetii*, while the remaining 6 per cent was caused principally by *Fusarium culmorum* var. *leteius* Sher. and *Fusarium avenaceum* (Fr.) Sacc. These latter came principally from northern Wisconsin and Minnesota where corn is not generally grown. During that season no perithecia of *Gibberella saubinetii* could be found on any of the corn refuse in these northern sections, while in the Corn Belt, perithecia of this fungus were very abundant. In 1920, a large number of scabbed wheat heads were collected from the States included in the Corn Belt and sent to Madison. During this season 98 per cent of the scab was found to be caused by *Gibberella saubinetii*, while most of the remaining 2 per cent was caused by *Fusarium avenaceum*.

The isolation studies would indicate, therefore, that *Gibberella saubinetii* is the principal organism concerned. In fact, it probably is the only one of economic importance in the production of wheat scab in the United States and therefore has been used in all of the inoculation experiments.

Inoculation experiments with corn growing under field conditions have been conducted for a number of years at Bloomington, Ill. It has been reported by Holbert, Dickson, and Biggar (*9*) that germination was lowered, early growth retarded, relative vigor throughout the season reduced, and grain production lessened by inoculation with *Gibberella saubinetii*. Since that report, inoculation experiments have been conducted on a larger scale and methods of technique have been improved. Some of the later results are given by Holbert et al. (*12*[6]). The fundamental facts first reported (*9*) have held true. Summarized data on some of the largest and most significant experiments will be given here.

METHODS

Experiments[7] were conducted in 1921 at Bloomington, Ill., with a strain of *Gibberella saubinetii* isolated from wheat kernels. The inoculations were made with 3-day-old cultures grown on potato dextrose agar

[6] HOLBERT, J. R., and others. OP. CIT.

[7] Merle T. Jenkins, Scientific Assistant, Office of Cereal Investigations, rendered valuable assistance in conducting this experiment.

at 20° to 22° C. The seed was placed in an aqueous spore suspension of this organism for 10 minutes just previous to planting. The concentration of this spore suspension was such that when a drop was placed on a glass slide and covered with a cover glass, about 100 spores could be seen in one field of the microscope, using a 4 mm. objective.

Two strains of corn were used, both originally from Yellow Dent. One represented the most disease-resistant strain obtainable at the time and the other represented good, average seed corn that had had no special selection toward disease resistance. These selections were similar to those used in the experiments reported in Tables VI, VII, VIII, and IX.

TABLE VI.—*Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on fields previously cropped to clover and scabbed wheat, respectively, at Bloomington, Ill., in 1920*

PREVIOUS CROP—CLOVER

Plat No.	Resistant seed.			Plat No.	Susceptible seed.		
	Field stand.		Acre yield.		Field stand.		Acre yield.
	Number of stalks.	Per cent.			Number of stalks.	Per cent.	
			Bushels.				*Bushels.*
A 1	37	77.1	80.9	B 1	34	70.8	69.6
A 2	42	87.5	88.9	B 2	39	81.3	75.8
A 3	43	89.6	92.8	B 3	41	85.4	72.0
A 4	39	81.3	88.9	B 4	41	85.4	81.3
A 5	40	83.3	78.6	B 5	43	89.6	72.7
A 6	37	77.1	84.2	B 6	39	81.3	71.2
A 7	43	89.6	88.9	B 7	41	85.4	72.7
A 8	40	83.3	77.0	B 8	39	81.3	72.7
A 9	39	81.3	88.8	B 9	42	87.5	85.2
A 10	43	89.6	91.2	B 10	39	81.3	67.3
Average	40.3	84.0	86.0	Average	39.8	82.9	74.1
C 1	48	100.0	79.3	D 1	40	83.3	68.0
C 2	45	93.8	80.2	D 2	40	83.3	78.8
C 3	39	81.3	78.6	D 3	41	85.4	67.3
C 4	45	93.8	94.4	D 4	34	70.8	69.6
C 5	40	83.3	81.0	D 5	40	83.3	62.6
C 6	45	93.8	78.8	D 6	36	75.0	63.4
C 7	40	83.3	77.8	D 7	38	79.2	73.6
C 8	41	85.4	90.4	D 8	41	85.4	80.6
C 9	41	85.4	80.1	D 9	40	83.3	76.7
C 10	42	87.5	81.7	D 10	40	83.3	69.6
Average	42.6	88.8	82.2	Average	39.0	81.3	71.0
E 1	43	89.6	72.0	F 1	36	75.0	69.3
E 2	46	95.8	80.4	F 2	42	87.5	73.6
E 3	41	85.4	84.0	F 3	45	93.8	85.7
E 4	43	89.6	88.7	F 4	39	81.3	70.8
E 5	44	91.7	82.9	F 5	40	83.3	70.8
E 6	40	83.3	77.2	F 6	39	81.3	70.0
E 7	38	79.2	83.6	F 7	39	81.3	71.2
E 8	39	81.3	84.3	F 8	37	77.1	72.8
E 9	40	83.3	87.4	F 9	35	72.9	70.4
E 10	41	85.4	88.1	F 10	40	83.3	79.9
Average	41.5	86.5	82.9	Average	39.2	81.7	73.5
General average.	41.5	86.4	83.7	General average.	39.3	81.9	72.9

TABLE VI.—*Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on fields previously cropped to clover and scabbed wheat, respectively, at Bloomington, Ill., in 1920*—Continued

PREVIOUS CROP—SCABBED WHEAT

Plat No.	Resistant seed. Field stand. Number of stalks.	Resistant seed. Field stand. Per cent.	Resistant seed. Acre yield.	Plat No.	Susceptible seed. Field stand. Number of stalks.	Susceptible seed. Field stand. Per cent.	Susceptible seed. Acre yield.
			Bushels.				*Bushels.*
A 1	39	81. 3	65. 6	B 1	34	70. 8	54. 5
A 2	45	93. 8	73. 5	B 2	37	77. 1	49. 5
A 3	46	95. 8	81. 4	B 3	37	77. 1	43. 6
A 4	41	85. 4	77. 0	B 4	34	70. 8	55. 1
A 5	38	79. 2	76. 2	B 5	34	70. 8	61. 0
A 6	43	89. 6	80. 1	B 6	44	91. 7	66. 5
Average	42. 0	87. 5	75. 6	Average	36. 7	76. 4	55. 0
C 1	42	87. 5	67. 6	D 1	40	83. 3	63. 9
C 2	44	91. 7	56. 0	D 2	38	79. 2	62. 0
C 3	39	81. 3	68. 7	D 3	42	87. 5	45. 1
C 4	38	79. 2	73. 0	D 4	44	91. 7	50. 8
C 5	41	85. 4	74. 5	D 5	37	77. 1	59. 4
C 6	43	89. 6	69. 8	D 6	39	81. 3	53. 2
Average	41. 2	85. 8	68. 3	Average	40. 0	83. 3	55. 7
E 1	42	87. 5	76. 8	F 1	36	75. 0	47. 6
E 2	46	95. 8	73. 3	F 2	41	85. 4	51. 8
E 3	45	93. 8	74. 7	F 3	45	93. 8	55. 1
E 4	40	83. 3	66. 8	F 4	43	89. 6	50. 4
E 5	47	97. 9	74. 4	F 5	43	89. 6	52. 2
E 6	42	87. 5	69. 1	F 6	39	81. 3	58. 2
Average	43. 7	91. 0	72. 5	Average	41. 2	85. 8	52. 6
General average	42. 3	88. 1	72. 1	General average	39. 3	81. 8	54. 4

TABLE VII.—*Summary of data given in Table VI on the relation of previous cropping to the yield of corn grown from disease-resistant and disease-susceptible seed at Bloomington, Ill., in 1920*

Previous crop.	Acre yield of corn grown from disease-resistant seed.	Acre yield of corn grown from disease-susceptible seed.
	Bushels.	*Bushels.*
Clover (relatively clean soil)	83. 7±0. 8	72. 9±0. 8
Scabby wheat (infested soil)	72. 1±0. 9	54. 4±1. 1
Difference	11. 6±1. 2	18. 5±1. 4

TABLE VIII.—*Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on a field of newly broken bluegrass sod and on a field previously cropped with scabbed wheat, at Bloomington, Ill., in 1921*

PREVIOUS CROP—BLUEGRASS SOD

Plat No.	Resistant seed. Field stand. Number of stalks.	Resistant seed. Field stand. Per cent.	Resistant seed. Acre yield.	Plat No.	Susceptible seed. Field stand. Number of stalks.	Susceptible seed. Field stand. Per cent.	Susceptible seed. Acre yield.
			Bushels.				*Bushels.*
A 1	114	95.0	65.7	B 1	115	95.8	56.3
A 2	114	95.0	61.3	B 2	110	91.7	51.1
A 3	113	94.2	57.0	B 3	108	90.0	45.2
A 4	119	99.2	59.6	B 4	110	91.7	51.9
A 5	101	84.2	50.8	B 5	108	90.0	47.8
A 6	105	87.5	48.7	B 6	110	91.7	54.5
A 7	100	83.3	46.0	B 7	103	85.8	51.3
A 8	107	89.2	54.3	B 8	106	88.3	47.4
A 9	114	95.0	62.4	B 9	115	95.8	46.0
Average	109.7	91.4	56.2	Average	109.4	91.2	50.2
C 1	117	97.5	60.9	D 1	117	97.5	52.4
C 2	116	96.7	49.8	D 2	114	95.0	58.5
C 3	119	99.2	60.7	D 3	116	96.7	52.8
C 4	119	99.2	56.9	D 4	115	95.8	48.4
C 5	120	100.0	48.0	D 5	116	96.7	49.8
C 6	115	95.8	52.2	D 6	116	96.7	48.2
C 7	119	99.2	53.5	D 7	117	97.5	41.4
C 8	116	96.7	51.9	D 8	109	90.8	52.1
C 9	120	100.0	58.7	D 9	116	96.7	53.7
Average	117.9	98.3	54.7	Average	115.1	95.9	50.8
E 1	117	97.5	67.0	F 1	108	90.0	66.8
E 2	119	99.2	67.4	F 2	86	71.7	46.1
E 3	119	99.2	67.7	F 3	109	90.8	63.4
E 4	118	98.3	51.3	F 4	104	86.7	52.6
E 5	117	97.5	60.0	F 5	100	83.3	47.6
E 6	116	96.7	56.3	F 6	105	87.5	58.7
E 7	114	95.0	59.6	F 7	107	89.2	54.6
E 8	110	91.7	58.5	F 8	101	84.2	66.3
E 9	116	96.7	45.0	F 9	109	90.8	61.3
Average	116.2	96.9	59.2	Average	103.2	86.0	57.5
General average	114.6	95.5	56.7	General average	109.2	91.0	52.8

TABLE VIII.—*Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on a field of newly broken bluegrass sod and on a field previously cropped with scabbed wheat, at Bloomington, Ill., in 1921*—Continued

PREVIOUS CROP—SCABBED WHEAT

Plat No.	Resistant seed. Field stand. Number of stalks.	Resistant seed. Field stand. Per cent.	Resistant seed. Acre yield.	Plat No.	Susceptible seed. Field stand. Number of stalks.	Susceptible seed. Field stand. Per cent.	Susceptible seed. Acre yield.
			Bushels.				*Bushels.*
A 1	114	95.0	52.4	B 1	104	86.7	48.4
A 2	119	99.2	59.3	B 2	106	88.3	50.9
A 3	114	95.0	48.9	B 3	113	94.2	38.8
A 4	115	95.8	46.4	B 4	112	93.3	30.6
A 5	114	95.0	51.3	B 5	114	95.0	31.6
A 6	113	94.2	50.8	B 6	110	91.7	37.0
A 7	116	96.7	61.7	B 7	109	90.8	50.6
A 8	112	93.3	58.7	B 8	111	92.5	43.7
A 9	115	95.8	50.2	B 9	111	92.5	36.6
Average	114.7	95.6	53.3	Average	110.0	91.7	40.9
C 1	115	95.8	67.3	D 1	115	95.8	49.5
C 2	116	96.7	55.0	D 2	102	85.0	47.3
C 3	112	93.3	51.0	D 3	98	81.7	42.8
C 4	119	99.2	50.2	D 4	108	90.0	39.5
C 5	114	95.0	55.4	D 5	113	94.2	41.2
C 6	117	97.5	50.4	D 6	112	93.3	45.6
C 7	119	99.2	57.0	D 7	116	96.7	40.1
C 8	115	95.8	51.3	D 8	114	95.0	48.2
C 9	120	100.0	46.0	D 9	115	95.8	43.2
Average	116.3	96.9	53.7	Average	110.3	91.9	44.2
E 1	118	98.3	60.2	F 1	99	82.5	52.6
E 2	118	98.3	66.6	F 2	105	87.5	49.7
E 3	117	97.5	46.1	F 3	106	88.3	44.9
E 4	114	95.0	49.7	F 4	106	88.3	51.1
E 5	114	95.0	57.8	F 5	105	87.5	43.9
E 6	118	98.3	54.3	F 6	106	88.3	36.2
E 7	113	94.2	59.3	F 7	95	79.2	41.0
E 8	111	92.5	50.4	F 8	95	79.2	45.2
E 9	116	96.7	49.6	F 9	109	90.8	42.3
Average	115.4	96.2	54.9	Average	102.9	85.7	45.2
General average	115.5	96.2	54.0	General average	107.7	89.8	43.4

TABLE IX.—*Summary of data given in Table VIII on the relation of previous cropping to the yield of corn grown from disease-resistant and disease-susceptible seed at Bloomington, Ill., in 1921*

Previous crop.	Acre yield of corn grown from disease-resistant seed.	Acre yield of corn grown from disease-susceptible seed.
	Bushels.	*Bushels.*
Virgin bluegrass sod (clean soil)	56.7±1.0	52.8±0.7
Scabbed wheat (infested soil)	54.0±1.2	43.4±1.0
Difference	2.7±1.6	9.4±1.2

The plats were located on a uniform piece of virgin, bluegrass sod with good drainage. The size of these plats was 4 rows wide by 10 hills long. Alternate with every plat planted with inoculated seed was a plat planted with uninoculated seed. As shown in Table X, these plats were replicated a number of times for both the disease-resistant and disease-susceptible selections. All the hills were 42 inches apart each way and were planted at the rate of 3 kernels per hill. As in all the other corn experiments, the plantings were made by hand to insure accuracy. Field stand and yield were taken on the entire plat 4 rows wide by 10 hills long, a total of 40 hills with a perfect stand of 120 plants.

RESULTS

The field stand, the number of vigorous plants, and the acre yield were reduced in every instance by the inoculations, as shown in Table X. The reductions in yield were small, averaging 3 per cent, when seed of disease-resistant corn was used. A large decrease in yield, averaging 18 per cent, resulted from inoculating seed of disease-susceptible corn, which is representative of the corn in most general commercial use. By following the recommendations given by Holbert and Hoffer (*10*), and Holbert et al.,[8] seed corn that is disease-resistant to a considerable degree can be obtained by the average farmer, and thus at least part of the loss due to this organism can be avoided. In fact, disease-resistant seed is already being used, and is gaining in favor very rapidly in many parts of the Corn Belt.

The reductions in yield from susceptible corn were greater than the reductions in stand and were more comparable to the reduction in percentage of vigorous plants. This relationship in respect to vigorous plants has been previously pointed out by Holbert et al. (*12*). These experiments, as well as others previously reported, show that *Gibberella saubinetii*, isolated from infected wheat, is an active parasite on corn. The organism has not been found to go up through the stalk during the active growing period, but apparently confines its attack to the underground parts of the seedling, thus decreasing stand and plant vigor. This results in reduced resistance to later invasions by other parasites and in low yields of corn of poor quality.

[8] HOLBERT, J. R. and others. OP. CIT.

TABLE X.—*Data showing the field stands, percentages of vigorous plants, and acre yield from disease-susceptible and disease-resistant strains of Yellow Dent corn uninoculated and inoculated with Gibberella saubinetii, when grown on bluegrass sod at Bloomington, Ill., in 1921*

Character of corn.	Seed inoculation.	Number of replications.	Number of plants.	Percentage of field stand.	Percentage of vigorous plants.	Acre yield.	Reduction in yield caused by inoculation.	
						Bushels.	*Bushels.*	*Per ct.*
Disease-susceptible.	Control	8	888	92.5	79.6	59.1±1.1	.....	.....
	G. saubinetii	4	386	80.4	59.4	48.6±1.9	10.5±2.2	17.8
Disease-susceptible.	Control	8	878	91.5	75.8	54.6±1.4	.....	.....
	G. saubinetii	4	385	80.2	58.9	44.7±1.3	9.9±1.9	18.1
Disease-resistant.	Control	28	3,300	98.2	90.4	74.2±0.9	.....	.....
	G. saubinetii	14	1,572	93.6	80.9	71.6±1.3	2.6±1.6	3.5
Disease-resistant.	Control	28	3,259	97.0	87.2	72.1±0.9	.....	.....
	G. saubinetii	14	1,556	92.6	78.2	70.4±1.1	1.7±1.4	2.4

CROP ROTATION FOR THE CORN BELT

It is evident that wheat should not follow corn in a rotation if wheat scab is to be held in check. Even though the stalks are removed and the field is plowed, some corn stubble will not be turned under and some will be pulled up again by the harrow. The wheat-scab infection in this case will be much less than if most of the cornstalks were left on the field, but it will be higher than it would be if there had been no corn preceding the wheat.

In a four-year rotation, including one year of corn and one year of wheat, there would be considerable advantage from the standpoint of controlling wheat scab and corn rootrots in arranging the rotation so that the wheat will neither directly precede nor follow the corn crop. Other small grains such as oats or barley, soybeans, clover or other legumes, or timothy, would be desirable to use as the intervening crops, as they are either immune or generally only slightly susceptible to the attacks of *Gibberella saubinetii*.

In large parts of the Corn Belt it may be desirable to grow corn more frequently than one year in four. In that case it seems best to use a five-year rotation with two years of corn in succession. For the other three years the same plan as above can be followed.

Some people insist on using a rotation of two years' corn in every four, with wheat as one of the intervening crops. In that case the wheat should immediately precede rather than follow the corn crop, taking care to use the best disease-resistant seed corn obtainable. It seems doubtful, however, whether a crop rotation of 50 per cent corn can be recommended from the standpoint of permanent crop improvement.

SUMMARY

Wheat scab was most severe where wheat followed corn in the rotation. The yield of corn susceptible to rootrot was considerably reduced where corn followed badly scabbed wheat in the rotation.

Gibberella saubinetii was the principal organism isolated from scabbed wheat heads. The same organism was found very abundantly on old corn-stalks.

When used as an inoculum on disease-susceptible corn this organism caused a considerable decrease in stand, general vigor, and yield.

Resistant strains of corn, field selected, well cured, and also selected on the germinator for vigor and freedom from disease, were injured to a less extent by inoculation with the wheat-scab organism. Such corn also did not suffer much reduction in yield when grown after scabbed wheat.

A crop rotation in which the wheat crop neither directly precedes nor follows the corn crop seems highly advantageous for the corn-wheat sections.

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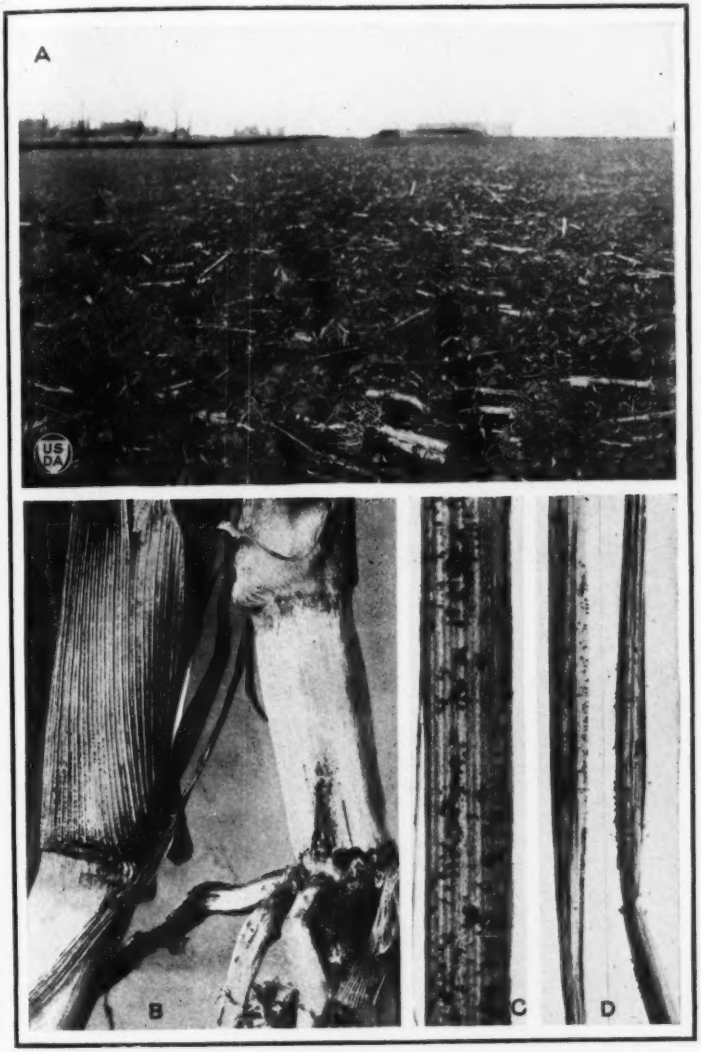
PLATE 1

Gibberella saubinetii on crop refuse

A.—Winter wheat sown on corn land. The cornstalks were broken down with a drag, after which the field was disked both ways and then plowed. Enough diseased stalks remained on the surface to carry scab to the entire wheat crop if weather conditions were favorable for the development of scab during and immediately following the blossom period.

B, C.—Perithecia of *Gibberella saubinetii* on cornstalks and stubble left on the surface of the soil.

D.—Perithecia of *Gibberella saubinetii* on wheat stubble and straw which was found on the surface of the ground in the vicinity of a straw stack.


A
B
C
D

A
B
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PLATE 2

Corn root and stalk rot caused by *Gibberella saubinetii*

A, B.—The influence of seed inoculation with *Gibberella saubinetii* on the development of corn plants. A, Yellow Dent corn planted on clean land; seed inoculated with *G. saubinetii* at time of planting. B, control. Note the difference in height of plants of the same age in inoculated and control rows.

C.—Plowing under a field of Marquis wheat ruined by scab. The yield of disease-susceptible corn planted on this field the following season was greatly reduced, whereas the disease-resistant corn gave fair yields.

MEASUREMENT OF LINKAGE VALUES[1]

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The demonstration that Mendelian characters are inherited in groups that correspond to the chromosomes has been followed by much intensive work directed to the determining of the relative position of the genes in the individual chromosomes.

If two Mendelian characters are shown to be correlated in transmission their genes are assumed to lie in the same chromosome, and are said to be linked. The early idea that the linkage relations might exhibit a numerical regularity comparable to Mendelian ratios has been abandoned. There is now much evidence to show that the degree of association of genes assumed to lie in the same chromosome is profoundly influenced by environmental factors.

Since closely related progenies frequently show widely divergent crossover ratios for the same pair of characters, a high degree of accuracy in measuring the ratio may seem to be of little value. But in all work that aims to test or to extend knowledge of the interrelations of characters it is of the greatest importance to determine whether any two characters are inherited independently or show a low degree of linkage.

When the linkage is loose a large number of individuals is required to establish the linkage. In all such cases it is necessary to be sure that the method used for demonstrating the relationship of two characters is accurate. If the method is such that a correlation is indicated where none exists, large numbers will only inspire confidence in a spurious linkage.

The most generally used method of determining the linkage relations of two characters is to cross individuals of the F_1 with individuals that are homozygous for the recessive allelomorphs of both characters. The sum of the individuals representing the two crossover or nonparental combinations expressed as a percentage of the total population is then taken as representing the percentage of the gametes in which crossing-over has taken place. This is undoubtedly the most simple and direct method, for if dominance is complete each back-crossed individual indicates directly the nature of the F_1 gamete that entered into its composition.

With many plants this method of back-crossing can not be used. Often the technique is difficult and it is impossible to obtain back-crossed individuals in sufficient numbers to afford reliable averages. In locating new recessive characters by the back-cross method there is the loss of a generation necessary to obtain a double recessive stock. In other instances double recessive individuals are weak and it is difficult to maintain the stock. Because of these difficulties in determining linkage values by the direct method many investigators have come to use F_2 populations.

[1] Received for publication Feb. 1, 1924.

A number of methods have been proposed for determining the crossover ratio from an F_2 population, any of which will give accurate results for a theoretically perfect population. In practice, however, disturbing factors, such as differential death rates or indistinct classes frequently cause significant departures from a theoretically perfect population.

Disturbing factors are made manifest by departures from the expected Mendelian ratios for each character. Deviations from expected Mendelian ratios may be due to a variety of causes any of which may be without effect on the percentage of gametes in which crossingover has taken place, but which may influence the apparent percentage of crossingover as determined from zygotic ratios.

The results obtained by the different formulae are variously affected by departures from expected Mendelian ratio depending on the cause of the deviation, and there seems to be no single method that will give the most accurate results in all cases.

Although it may not be possible to determine with certainty the cause of any given departure from an expected Mendelian ratio, frequently there will be strong presumptive evidence in favor of some one cause, and when such is the case it should be of value to be able to choose a method that is not seriously affected by the disturbing factor. In the following pages the methods in most general use have been brought together and compared empirically as to their accuracy.

NATURE OF ABERRANT MENDELIAN RATIOS

Departures from expected Mendelian ratios may be due to irregularities in the behavior of the gametes or of the zygotes. Several types of gametic disturbances have been recognized, such as the formation of dominant and recessive gametes in unequal numbers, differential mortality, and selective fertilization. The effect on linkage relations is the same in all cases—that is, effective gametes bearing the dominant and recessive members of an allelomorphic pair are unequal in number.

Similarly, there may be a variety of ways in which one zygotic class is influenced adversely, but the general result is a differential mortality or a difference in the survival value of dominant and recessive individuals.

In addition to these two main causes of aberrations there is the slightly different case that results from mistakes in the classification of zygotes. The deviation is, of course, zygotic, but while the numbers representing one member of the character pair are increased at the expense of the other, it is not the same as a differential mortality, for if there is a linkage between the two character pairs a differential death rate in one character must necessarily affect the ratio of dominant to recessive in the other character, whereas mistakes in the classification of one character do not affect the ratio of dominant to recessive in the second character.

In discussing the various measures of linkage the following terminology will be used:

AB, Ab, aB, ab = the number of individuals in each of the four zygotic classes in a hybrid involving two character pairs Aa and Bb.

n = total number of individuals.

p = that part of the total number of gametes in which crossingover has taken place expressed as a decimal fraction.

$1-p$ = noncrossover gametes.

The percentage of crossingover is then $p \times 100$ and the crossover ratio is $\frac{p}{p} : \frac{1-p}{p}$.

MEASUREMENT OF LINKAGE BY THE BACK-CROSS METHOD

The back-cross method, sometimes called the direct method, may be used when the cross is of the nature *Aa Bb* × *aa bb*. The formula is:

$$p=\frac{Ab+aB}{n}$$

The probable error of this determination is usually given as:

$$E_p=.6745\sqrt{\frac{p\,(1-p)}{n}}$$

Even this approved method of determining the degree of linkage may not be entirely free from error when the Mendelian ratios do not agree with the expected.

Haldane states (*5*,[1] *p. 292*) that by the method of back-crossing "we automatically eliminate the effects of differential mortality due to one factor, or to both if they affect the mortality independently." This appears to be an error. Take a simple example with no linkage, if there is no differential mortality the four zygotic classes will be equal. If now in such a population 50 per cent of the recessive class of each character fails to survive, the observed classes will be in the proportion 4 *AB*, 2 *Ab*, 2 *aB*, 1 *ab*, indicating a linkage between dominants with 44 per cent crossing-over, 4/9=.44+.

If the Mendelian ratios are aberrant in only one character the linkage relations are not affected, but where there are departures in both, the per cent of crossover individuals will fail to indicate the true ratio of crossover to noncrossover gametes. If the extent of the error or the spurious linkage be designated *e*, then its value is $e=.5-r-s+2\,rs$ where r = the number of individuals recessive with respect to one character divided by the total number of individuals and s = the corresponding determination with respect to the other character.

If either r or $s=0.5$, e will equal 0.0—that is, there is no spurious linkage. If both r and s depart from 0.5 in the same direction there will be a spurious linkage between dominants. If r and s depart from 0.5 in opposite directions the spurious linkage will be between dominant and recessive.

To eliminate the effects of differential mortality on the measurements of linkage in back-crosses, Bridges (*2*, *p. 3*) proposed the method of balanced viability. The procedure is to average the results obtained from two original crosses in which the parental combinations are reversed, that is, *AABB*×*aabb* and *AAbb*×*aaBB*. This method practically eliminates the effects of differential mortality, either in gametes or zygotes and undoubtedly is the most desirable method to use whenever material for making the two crosses can be made available.

METHODS FOR DETERMINING LINKAGE VALUES FROM AN F_2 POPULATION

Three general methods have been proposed for measuring linkage values in an F_2 population. These are, Yule's coefficient of association, "Q," Emerson's method, "*P*," and Haldane's method "*T*."

[1] Reference is made by number (italic) to "Literature cited," p. 891.

YULE'S COEFFICIENT OF ASSOCIATION

The formula for Q is:

$$Q=\frac{Ab\times aB-AB\times ab}{Ab\times aB+AB\times ab}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where AB, Ab, aB, and ab represent the numbers of individuals in the four zygotic classes of an F_2 population. The probable error of Q is:

$$E_Q=.6745\times\frac{1-Q^2}{2}\sqrt{\frac{1}{AB}+\frac{1}{Ab}+\frac{1}{ab}+\frac{1}{aB}}$$

By reference to a table giving values of Q for the various values of p, the value of p corresponding to the observed Q is assumed to be the p of the observed population.

A short table of the values of Q for integral gametic ratios where dominants are linked was given by the author (*3*). It was pointed out by Bridges (*1*) that a different table was needed when dominant and recessive are linked and for accurate determinations it is, of course, necessary to resort to interpolations. These difficulties may be obviated by using the following formula:

$$p^2=\frac{\sqrt{4-2Q(Q-1)}-2}{2Q}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

To make this formula applicable when dominants are linked it is necessary to retain the arrangement of the zygotic classes in the following order, $\frac{(Ab\times aB)-(AB\times ab)}{(Ab\times aB)+(AB\times ab)}$. When the linkage is between dominants and Q is negative, p will be greater than 0.5 and will represent the noncrossover group.

In a back-cross the coefficient of association also may be used to measure the degree of linkage and the result is not affected by differential death rates. In back-crosses $p=\frac{Q-1+\sqrt{1-Q^2}}{2Q}$.

EMERSON'S METHOD

The second method for evaluating p was proposed by Emerson (*4*). Stated in terms of p and $1-p$ instead of the r and s of the original formula, and taking $AB+ab$ as the crossover classes, the determination becomes

$$p=\sqrt{\frac{2\,(AB+ab)}{n}-1}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

No formula for the probable error of this method has been suggested, but the formula for the probable error of a ratio given under the back-cross method should provide a fair measure of the reliability of results. This method has the merit of extreme simplicity, but as Emerson pointed out it becomes unreliable when there are wide departures from expected Mendelian ratios. The method has been extended by Woodworth (*7*) to include multiple-factor characters. In such cases the use of p and $1-p$ for the crossover and noncrossover gametes instead of r and s

results in a very material simplification. Thus Woodworth's formula (2) for two characters segregating in the ratios 3 : 1 and 15 : 1 which is given as $12r^2+11\ (s^2+2\ rs)\ :\ 3\ r^2+4\ (s^2+2\ rs)\ :\ s^2+2\ rs\ :\ r^2$, becomes $11+p^2\ :\ 4-p^2\ :\ 1-p^2\ :\ p^2$, and the formula for evaluating p from an observed zygotic distribution becomes, $p=\sqrt{\frac{8\ (AB+ab)}{n}-5{\cdot}5}$

Formulae for calculating p for some of the zygotic distributions most frequently encountered are given below:

$$3:1 \text{ and } 1:1 \quad p=\frac{2\ (Ab+aB)}{n}-.5$$

$$63:1 \text{ and } 3:1 \quad p^2=\frac{32\ (AB+ab)}{n}-23.5$$

$$15:1 \text{ and } 15:1 \quad p^2=\frac{32\ (AB+ab)}{n}-28$$

$$15:1 \text{ and } 3:1 \quad p^2=\frac{8\ (AB+ab)}{n}-5{\cdot}5$$

$$9:7 \text{ and } 3:1 \quad p^2=\frac{16\ (AB+ab)-7n}{6n}$$

$$9:7 \text{ and } 9:7 \quad p^2=\frac{32\ (AB+ab)-14n}{9n}$$

$$9:7 \text{ and } 15:1 \quad p^2=\frac{32\ (AB+ab)-17n}{3n}$$

HALDANE'S METHOD

The method proposed by Haldane may be written in the form:

$$p=\sqrt{T}=\sqrt{\frac{3t_at_1+(2+t_a)t_4}{2+4t_a}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where

$$t_1=\frac{4AB}{n}-2$$

$$t_4=\frac{4ab}{n}$$

$$t_a=\frac{t_1+t_4}{2}$$

t_a is an approximate value and when the observed classes are wide departures from expected Mendelian ratios it is necessary to make a further approximation by substituting T for t_a in formula (4).

The method used in deriving the formula is to determine the most probable value of p^2 for an observed population where the departures from expected Mendelian ratios are the result of errors of sampling. The probable error is given by Haldane (5, p. 295) as:

$$E_p=.477\sqrt{\frac{(2+p^2)\ (1-p^2)}{(1+2p^2)\ n}}.$$

Haldane states (*5, p. 294*) that the method gives the same value for p as Yule's coefficient of association. This is, of course, true for a perfect distribution and it appears to hold also where the Mendelian ratio of only one of the characters is disturbed through a differential mortality of either gametes or zygotes, but where both characters are affected the two methods do not give identical results. Take for example a case with no linkage, that is $p=.5$, in which ½ the recessive gametes of both characters are not effective. The observed zygotic classes would be 64: 8: 8 : 1, $n=81$, $Q=0$, giving $p=.5$.

Haldane's formula gives $p=.7111$ as the first approximation. As a result of ten successive substitutions of T for t_4 the value of p is reduced to 0.6626 the last substitution producing no change in the first four decimal places. It is clear that 0.5 is not the asymptote.

COMPARISON OF METHODS

In determining the per cent of crossingover from an F_2 population it is necessary to bear in mind that a value of p which gives a constructed population in closest agreement with the observed population may not be the correct value or the best that it is possible to find.

Before any attempt can be made to evaluate p it is necessary to make some assumption regarding the factorial composition of the characters involved. If these assumptions are wrong any formula will lead, of course, to an erroneous value of p. It follows, further, that if there are departures from the expected Mendelian ratios, whether due to chance, selective fertilization, differential death rates or mistakes in classification, these departures may introduce errors into the calculation of p when populations with perfect Mendelian ratios are used as criteria. A method which would determine p by reference to a population with the observed instead of the theoretical Mendelian ratios should be free from this defect.

Of the proposed methods the coefficient of association, Q, is the one that most nearly meets this requirement. Since Q is based on the relation that exists between the product of the two crossover classes as compared with the product of the two noncrossover classes it is unaffected by zygotic changes in Mendelian ratios.

Q is slightly affected by mistakes in classification, and by differential fertilization, but the most frequent cause of departures from Mendelian ratios is a differential death rate of zygotes and this has no effect on the value of Q. That Q has decided limitations as a measure of correlation should not affect its use as a measure of linkage. There is, however, one serious drawback to the use of Q. When any one of the classes is small, changes in this class produce a disproportionately large effect on the value of Q. When one class is 0 and Q equals 1, it may be open to argument whether the correlation is or is not perfect, but certainly it does not follow that the linkage is complete.

When dominant and recessive are linked and the population is small failure to recover the double recessive class may result from errors of sampling. In such cases p may be calculated from the other three classes as follows:

$$p=\sqrt{\frac{2\,AB-2\,(Ab+aB)}{2\,AB+(Ab+aB)}}$$

Similarly, if there is difficulty in distinguishing any two classes as, for example, aB and ab p may be calculated from the ratio of AB to Ab or AB to the total

$$p=\sqrt{\frac{AB-2\,Ab}{AB+Ab}} \text{ or } p=\sqrt{\frac{4\,AB-2\,n}{n}}$$

χ^2 AS A MEASURE OF LINKAGE

One of the most fundamental determinations in connection with linkage problems is to discriminate between a low linkage value and independent inheritance. This is done by calculating the χ^2 of the observed population from a theoretical population of the same size with no linkage.

If the theoretical population is constructed in accordance with the expected Mendelian ratio the result will be erroneous unless the observed population conforms exactly to the expected Mendelian ratios. Any irregularity in the behavior of the individual characters will increase χ^2 and may indicate a linkage when none exists.

It would seem that the correct procedure should be to construct the theoretical population from the observed instead of the expected Mendelian ratios.

A theoretical population, $A'B'$, $A'b'$, $a'B'$ and $a'b'$ of the same size and having the same Mendelian ratios may be constructed as follows:

$$a'b' = \frac{(aB+ab)\times(Ab+ab)}{n}$$

$$a'B' = \frac{(aB+ab)\times(AB+aB)}{n}$$

$$A'b' = \frac{(AB+Ab)\times(Ab+ab)}{n}$$

$$A'B' = \frac{(AB+Ab)\times(AB+aB)}{n}$$

The observed distribution is compared with the theoretical distribution and χ^2 is determined as follows:

$$\chi^2 = \frac{(AB-A'B')^2}{A'B'} + \frac{(Ab-A'b')^2}{A'b'} + \frac{(aB-a'B')^2}{a'B'} + \frac{(ab-a'b')^2}{a'b'}$$

The degree of confidence to be placed in the observed departure is indicated in the following table:

χ^2	Odds against the deviation being due to chance[1].
1	.2 to 1
2	.7 to 1
3	1.6 to 1
4	2.8 to 1
5	4.8 to 1
6	8.0 to 1
7	12.9 to 1
8	20.7 to 1
9	33.1 to 1
10	52.9 to 1
11	84.3 to 1
12	134.4 to 1
13	214.7 to 1
14	343.2 to 1
15	549.4 to 1
16	880.8 to 1
17	1,413.4 to 1
18	2,271.7 to 1
19	3,662.0 to 1
20	5,881.4 to 1

[1] Calculated from the values of P given in Pearson's "Tables for Statisticians and Biometricians," Table XII (6).

Since the different methods of calculating crossover ratios are variously affected when the Mendelian ratios show departures from the expected, it may be of value to determine empirically how Mendelian deviations of the various kinds affect the results obtained by the different methods.

It would be of great interest to have the effects of Mendelian deviations on the various methods investigated by a competent mathematician, but until this is done a comparison of examples may be of some assistance.

Deviations from expected Mendelian ratios fall into three groups:

(1) Effective gametes of the recessive and dominant classes are not produced in equal numbers—Differential mortality of gametes.

(2) Individuals showing the dominant or recessive character may not survive in equal percentages—Differential mortality of zygotes.

(3) Individuals showing the dominant or recessive character may be wrongly classified as belonging to the opposite class.

In each of the above the departure may affect one or both characters.

Back-crosses and straight F_2 populations will be considered separately. In the case of a back-cross (1) and (2) produce the same result.

It should be kept in mind that the differential death rates with which we are concerned and the effect of which we wish to eliminate are those in which one member of an allelomorphic pair, considered without respect of other Mendelian characters, has a higher death rate than its mate. Death rates due to incompatible gametic or zygotic combinations, may be distinguished from linkage by the method of balanced inviability proposed by Bridges.

Table I shows the effect of the various forms of Mendelian aberrations on a back-crossed population. It will be noted that in many cases a formula gives the correct result when $p = .5$ and does not give the correct result when there is a linkage.

Table II compares the accuracy of the three methods in F_2 populations where there is no linkage.

It appears that Q gives the correct result except where one of the classes is not represented.

T likewise gives the correct result with either gametic or zygotic disturbances provided only one of the characters is affected. Where both characters depart from the expected ratio, either gametically or zygotically, a linkage is indicated where none exists.

With departures from the expected of any kind P indicates a linkage, but in cases where the double recessive class is not recovered, P indicates but a slight departure from no linkage while both Q and T indicate perfect linkage.

Table III makes similar comparisons where there is linkage with 25 per cent crossingover. It appears that Q gives the correct result in all cases where the disturbance is zygotic except in mistaken classification and failure to recover the double recessive class. In the latter instance P is only slightly affected. P also has a slight advantage in the case where recessives are misclassified as dominant and the linkage is between dominants.

When the disturbance is gametic there is very little difference between Q and T though Q has a slight advantage in five of the six examples.

TABLE I.—*Measures of linkage in a back-cross*

(1) No linkage, i. e., $p = .5$

	Zygotic classes.					Observed value of p.	
	AB.	*Ab.*	*aB.*	*ab.*	Total.	$\frac{Ab+aB}{n}$	*Q.*
Perfect population	1	1	1	1	4	0. 5000	0. 5000
25 per cent *a* gametes not effective	4	4	3	3	14	. 5000	. 5000
25 per cent *a* and *b* gametes not effective	16	12	12	9	49	. 4898	. 5000
25 per cent *a* and *B* gametes not effective	12	16	9	12	49	. 5102	. 5000
25 per cent *a* zygotes wrongly classified as *A*	5	5	3	3	16	. 5000	. 5000

(2) Dominants linked, 25 per cent crossingover, i. e., $p = .25$

Perfect population	3	1	1	3	8	0. 2500	0. 2500
25 per cent *a* gametes not effective	12	4	3	9	28	. 2500	. 2500
25 per cent *a* and *b* gametes not effective	16	4	4	9	33	. 2424	. 2500
25 per cent *a* and *B* gametes not effective	36	9	16	36	97	. 2577	. 2500
25 per cent *a* zygotes wrongly classified as *A*	13	7	3	9	32	. 3125	. 2976

TABLE II.—*Comparison of methods of measuring linkage in F_2*

No linkage, i. e., $p = .5$

	Zygotic classes.					Observed value of p.		
	AB.	*Ab.*	*aB.*	*ab.*	Total.	*Q.*	*P.*	*T.*
Perfect population	9	3	3	1	16	0. 5000	0. 5000	0. 5000
25 per cent *a* gametes not effective	120	40	27	9	196	. 5000	. 5624	. 5000
25 per cent *a* and *b* gametes not effective	1, 600	360	360	81	2, 401	. 5000	. 6336	. 5260
25 per cent *a* and *B* gametes not effective	1, 320	640	297	144	2, 401	. 5000	. 4685	. 4798
25 per cent *a* zygotes die.	12	4	3	1	20	. 5000	. 5477	. 5000
25 per cent *a* and *b* zygotes die	144	36	36	9	225	. 5000	. 6000	. 5133
25 per cent *a* and *B* zygotes die	108	48	27	12	195	. 5000	. 4804	. 4886
25 per cent *a* zygotes called *A*	39	13	9	3	64	. 5000	. 5590	. 5000
Double recessive class not recovered	9	3	3	0	15	. 0000	. 4472	. 0000

TABLE III.—*Comparison of methods for measuring linkage in F_2, 25 per cent crossing-over*

	Nature of linkage.	Zygotic classes.					Value of *p*.		
		AB.	*Ab*.	*aB*.	*ab*.	Total.	*Q*.	*P*.	*T*.
Perfect population...	*D* and *R*[a]..	33	15	15	1	64	0.2500	0.2500	0.2500
	D and *D*...	41	7	7	9	.....	.2500	.2500	.2500
25 per cent *a* gametes not effective.	*D* and *R*...	424	216	135	9	784	.2377	.5997	.2336
	D and *D*...	552	88	63	81	784	.2432	.2159	.2417
25 percent *a* and *b* gametes not effective.	*D* and *R*...	5,440	1,944	1,944	81	9,409	.2263	.4166	.2349
	D and *D*...	7,488	792	792	729	9,801	.2359	.1773	.2188
25 per cent *a* and *B* gametes not effective.	*D* and *R*...	4,824	3,456	1,377	144	9,801	.2496	.1174	.2288
	D and *D*...	5,976	1,408	729	1,296	9,409	.2498	.2612	.2605
25 per cent *a* zygotes die.	*D* and *R*...	132	60	45	3	240	.2500	.3536	.2500
	D and *D*...	164	28	21	27	240	.2500	.2308	.2500
25 per cent *a* and *b* zygotes die.	*D* and *R*...	528	180	180	9	897	.2500	.4442	.2619
	D and *D*...	656	84	84	81	905	.2500	.2071	.2416
25 per cent *a* and *B* zygotes die.	*D* and *R*...	396	240	135	12	783	.2500	.2053	.2415
	D and *D*...	492	112	63	108	775	.2500	.2595	.2582
25 per cent *a* called *A*.	*D* and *R*...	147	61	45	3	256	.2600	.4146	.2644
	D and *D*...	171	37	21	27	256	.2742	.2605	.2808
Double recessive not recovered.	*D* and *R*...	33	15	15	0	63	.0000	.2182	.0000

SUMMARY

Some measure of linkage is necessary in all studies of the association between characters and chromosomes.

Back-crossing the F_1 on double recessives affords the most direct measure but with many plants this method can not be used. There is also a slight though usually unimportant error in the use of this method when the Mendelian ratios depart from the expected.

Three methods have been proposed for measuring linkage in the F_2:

(1) Yule's coefficient of association, *Q*.

(2) Emerson's method, *P*.

(3) Haldane's method, *T*.

All of these give the correct result with a perfect population, but are variously affected by differential death rates of the gametes or zygotes, selective fertilization and mistakes in classification, which cause departures from expected Mendelian ratios.

Departures from expected Mendelian ratios are grouped into three classes and the effects of these departures on the accuracy of the three methods of measuring linkage in F_2 are investigated empirically.

No one method is found to be most accurate for all classes of departures.

Yule's coefficient of association, *Q*, most nearly meets the requirements of a general method and a formula is given for evaluating the degree of linkage from observed values of *Q*.

[a] D and R=dominant and recessive linked, D and D=dominants linked.

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